DESIGN AND SYNTHESIS OF NOVEL HETEROCYCLES AS POTENT ANTICANCER AND ANTI-INFECTIVE AGENTS

Thesis

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2009

Dedicated

to

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CERTIFICATE

This is to certify that the thesis entitled "**Design and synthesis of novel heterocylces as potent anticancer and anti-infective agents**" submitted to the Jawaharlal Nehru University, New Delhi in partial fulfillment of the requirement for the award of degree of Doctor of Philosophy, embodies the research work carried out by **Mr. Ravi Kumar** under my supervision at Central Drug Research Institute, Lucknow. The work presented in the thesis is original and has not been submitted so far to any other institute/university either in part or full for any degree or diploma.

(Dr. P. M. S. Chauhan)
Supervisor

CONTENTS

LIST OF ABBREVIATIONS

AcOH	: Acetic acid
Anal.	: Analysis
Anhyd	: Anhydrous
Aq.	: Aqueous
bs	: Broad singlet
^oC	: Degree Celsius
$CaCl_2$	: Calcium chloride
Calcd.	: Calculated
CC_{50}	: Cytotoxic concentration (50% suppression of cell line)
$CHCl_3$	: Chloroform
$CDCl_3$	: Deuterated chloroform
CH_3CN	: Acetonitrile
CH_3OH	: Methanol
CH_3OD	: Deuterated methanol
CS_2	: Carbondisulfide
d	: Doublet
dd	: Double doublet
DCM	: Dichloromethane
DHFR	: Dihydrofolate reductase
DIPEA	: N,N-Diisopropylethylamine
DMF	: N,N-dimethylformamide
DMSO	: Dimethyl sulfoxide
DMSO-d_6	: Deuterated dimethyl sulfoxide
DNA	: Deoxyribonucleic acid
EtOH	: Ethanol
Eq	: Equivalent
ESMS	: Electron spray mass spectroscopy
FAB	: Fast atom bombardment
g	: Gram(s)
h	: Hour(s)
HCl	: Hydrochloric acid
Hz	: Hertz(s)
^{1}HNMR	: Hydrogen nuclear magnetic resonance
IC_{50}	: Inhibitory concentration 50 %
IR	: Infrared
J	: Coupling Constant
K_2CO_3	: Potassium carbonate
$KHCO_3$	: Potassium bicarbonate
KBr	: Potassium bromide
kg	: Kilo gram
KOH	: Potassium hydroxide
L	: Liter
M^+	: Molecular ion peak
m-CPBA	: *meta*-Chloroperoxybenzoic acid
MeI	: Methyl iodide
MeOH	: Methanol
mg	: Milli gram(s)
MHz	: Mega hertz

MIC	: Minimum inhibition concentration
min.	: Minutes
mL	: Milli litre
mM	: Milli mole(s)
mp	: Melting point
MTT	: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
Na_2SO_4	: Sodium sulphate
NaH	: Sodium hydride
$NaHCO_3$	: Sodium bicarbonate
NaOH	: Sodium hydroxide
ng	: Nanogram(s)
pH	: Potential of Hydrogen
ppm	: Parts per million
rt	: Room temperature
s	: Singlet
SAR	: Structure activity relationship
t	: Triplet
TDW	: Triple distilled water
Temp.	: Temperature
TFA	: Trifluoroacetic acid
THF	: Tetrahydrofuran
TLC	: Thin layer chromatography
TMS	: Tetramethyl silane
Topo	: Topoisomerase(s)
v/v	: Volume/volume
µg	: Micro gram
µL	: Micro litre
µM	: Micro molar

Preface

Cancer, a group of as many as 200 diseases or disorders characterized by uncontrolled division of cells and the ability of these cells to invade other tissues. In the near past cancer has been regarded as a group of diseases affecting the more developed countries but now incidence of various forms of cancer are rapidly rising worldwide. Despite major breakthroughs in the area of medicine in the past century, better understanding of the disease and rationally targeted drugs, successful treatment of cancer still remains a significant challenge. Our goal in this dissertation is to synthesize novel molecules which can selectively inhibit cancer cells proliferation or kill them without affecting normal cells that limits the traditional chemotherapy of cancer. On the other hand, infectious diseases such as leishmaniasis and bacterial infections represent major global health problems of immense proportion and are the leading causes of death in tropical and subtropical regions of the world. Vaccine development against these infectious diseases seems unlikely because of the extreme antigenic variations exhibited by these parasites. Therefore, chemotherapy remains the only treatment option for controlling the infection once acquired. Unfortunately, either resistance has developed to the existing therapeutics or they are toxic to the human host and none of the therapeutic strategy involving these has proven consistently successful.

In the light of above facts and the vast biological importance of nitrogen containing heterocycles like triazine, indole, β-carboline, pyrimidine, 2-thiohydantoin, 2-aminoimidazolone and 2-aminoimidazole derivatives inspired us to synthesize these heterocyclic ring systems and their derivatives which may lead to strong drug candidate in the field of cancer and infectious disease like leishmaniasis and bacterial infections. This thesis consists of five chapters.

Chapter 1 presents the recent advances in β-carbolines as anticancer and anti-infective agents. This review compiles important developments in natural as well synthetic β-carbolines since January 1996. Among infectious diseases it covers antileishmanial, antimalarial and antifungal β-carbolines.

Chapter 2 describes design, synthesis and cytotoxicity evaluation of 1,3,5-triazine and tetrahydro-β-carboline hybrids as anticancer agents.

Chapter 3 describes the synthesis of 2-(pyrimidin-2-yl)-1-phenyl-2,3,4,9-tetrahydro-1*H*-β-carbolines as antileishmanial agents taking inspiration from recently isolated natural product annomontine.

Chapter 4 is divided into two parts (a) reviews the literature on marine 2-aminoimidazole, glycociamidine alkaloids and their synthetic analogues as new leads for drug development. While part (b) describes development of highly versatile, protecting group free synthesis of Isonaamine C, it's analogues and discovery of their antileishmanial and antibacterial potential.

Chapter 5 is also divided into two parts, (a) presents a one-pot chemoselective *S*-alkylation and acetylation of 2- thiohydantoins using the alkyl orthoformate-$ZnCl_2$-Ac_2O reagent system. Part (b) describes discovery of $SnCl_2.2H_2O$ as an efficient reagent for direct and selective oxidative desulfurization of phenylmethylene-2-thiohydantoins to corresponding hydantoins

General remarks

- All reactions were monitored by Thin layer chromatography (TLC) carried out on 2.5 × 5 cm plates coated with a 0.25 mm thickness of silica gel (60F-254) and visualization was accomplished by UV light or by I_2 or with $CeSO_4$ (1% in 1M H_2SO_4) and subsequent charring on hot plate.
- All evaporations were carried out under reduced pressure on Büchi rotary evaporator below 60 ºC unless otherwise specified.
- Silica gel (60-120), (100-200) and (230-400) mesh was used for column chromatography.
- Organic solvents are dried by standard procedure mentioned in "Textbook of Practical Organic Chemistry" by A. I. Vogel, Revised by Furniss, B. S.; Hannaford, A. J.; Smith, P. W. G. and Tache, A. R.; ELBS with Longmann, V edition.
- All dry reactions were carried out in oven dried glassware under nitrogen or argon atmosphere, using freshly dried solvents. Yields refer to pure compound after chromatography unless and otherwise stated.
- Common organic solvents for general use were purchased from E. Merck, Qualigens, Ranbaxy, and S. D. Fine, Spectrochem chemicals.
- All important reagents were purchased from Aldrich and Fluka chemical Co.
- NMR spectra were recorded on Bruker Avance DPX 200 FT, Bruker Robotics and Bruker DRX 300, Bruker Avance 400 Spectrometers at 200, 300 (^{1}H) and 50, 75 MHz (^{13}C). Experiments were recorded in $CDCl_3$, CD_3OD, DMSO-d_6 at 25 ºC. Chemical shifts were given in parts per million downfield from internal standard Me_4Si. Carbon atom types (CH, CH_2, CH_3) were determined by DEPT pulse sequence.
- The EI mass spectra were recorded on JMS 600H mass spectrometer at 70 ev. HRMS was calculated using PFK as internal standard. DART-HRMS was recorded on a JEOL-AccTOF JMS-T100LC mass spectrometer having a DART source.

- Optical rotations were determined on an Autopol III polarimeter using a 1 dm cell in chloroform/methanol as the solvent.
- Infrared spectra were recorded on Perkin-Elmer RX1 and FTIR-8210 PC Shimadzu Spectrophotometers either as KBr disc or neat and value expressed in cm^{-1}
- Elemental analysis was carried out on Carlo Erba-1108 (CHN) and Vario EL-III (CHNS-O) elemental analyser. C, H, N values were calculated as per the molecular weights C=12.01, H=1.008, N=14.007, O=15.999.

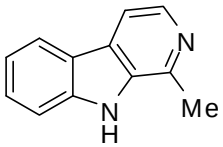
Chapter 1

A brief review on recent advances in β-carbolines as anticancer and anti-infective agents

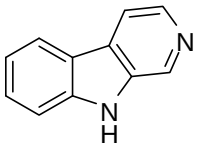
1.1 Introduction:

β-Carbolines are a large group of natural and synthetic indole alkaloids having common tricyclic pyrido[3,4-*b*]indole ring motif with varying degrees of aromaticity, some of which are widely distributed in nature including various plants, marine creatures, insects, foodstuffs, mammalians as well as human tissues and body fluids.[1,2] These compounds are of great interest due to their ability to act as agonist to benzodiazepine receptors, 5-hydroxy serotonin receptors[3] and intercalate into DNA,[4] to inhibit CDK, Topisomerase,[5] and monoamine oxidase enzymes.[6] In addition to this, their pharmacological properties include anxiolytic, hypnotic, anticonvulsant,[7] antiviral,[8] antiparasitic[9] and antimicrobial[10] activities.

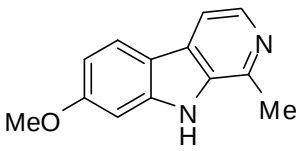
Chemically, these molecules can be classified according to the degree of saturation of their N-containing, six-membered ring i.e. fully unsaturated members are named as aromatic β-carbolines, whereas dihydro-β-carbolines is the name given to partially saturated members. Completely saturated members are known as tetrahydro-β-carbolines. β-carbolines can exist in four forms depending upon pH and solvent,[11]: the cationic, the neutral form, a zwitterionic (or an alternative quinine-type canonical form), and an anionic. These tricyclic β-carbolines usually contain several substituents both in the N-containing six membered ring and/or the indole ring in complex natural products having unique three dimensional arrangements of chiral functional groups for exhibiting specificity in protein binding and eliciting a specific biological response.



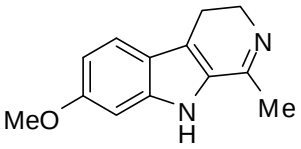
Harman (**1**)



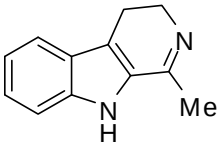
Norharman (**2**)



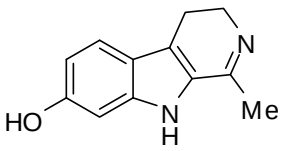
Harmine (**3**)



Harmaline (**4**)



Harmalan (**5**)



Harmalol (**6**)

Figure 1. Simple β-carboline alkaloids.

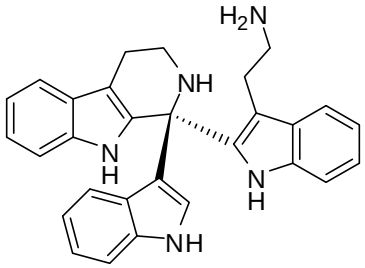
Earlier much emphasis had been focused on the spectroscopic, photophysical properties and the effects on the central nervous system (CNS), such as their affinity with benzodiazepine receptors (BZRs), 5-HT_{2A} and 5-HT_{2C} receptors[12-14] of the β-carbolines. Presence of two kinds of nitrogen in β-carbolines i.e. more basic pyridinic nitrogen and less basic pyrrolic one affects the photophyscial and photochemical properties of these alkaloids. Basicity of the pyrrolic nitrogen increases upon excitation[15,16] and is also affected by the presence of substituents in the tricyclic core.[17] However, recent interest in these alkaloids has been focused on their potent antitumor, and antiparasitic activities. Anticancer, antimalarial, antileishmanial and antifungal activities of β-carbolines are reviewed in this chapter.

1.2 β-carbolines as anticancer agents:

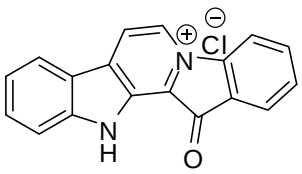
1.2.1 Natural products

1.2.1.1 Bengacarboline:

Bengacarboline (**7**),[18] a tetrahydro-β-carboline alkaloid with two indole unit connected at C-1 of the carboline nucleus, was isolated from chloroform fraction of Fijian ascidian sponge, *Didemnum* sp together with known cytotoxic β-carboline alkaloid fascaplysin (**8**).[19] It showed cytotoxic activity against a panel of 26 human tumor cell lines with mean IC_{50} value of 0.9 μg/mL. It has also inhibited the catalytic activity of topoisomerase II enzyme with IC_{50} value of 32 μM.



Bengacarboline (**7**)

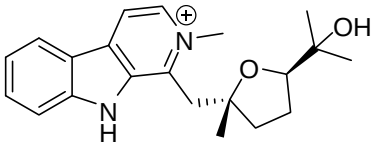


Fascaplysin (**8**)

1.2.1.2 Chrysotricine:

Chrysotricine (**9**) was isolated in trace amount from ethanolic extract of Chinese medicinal plant *Hedyotis chrysotricha*.[20] More than 20 plants of genus Hedyotis are

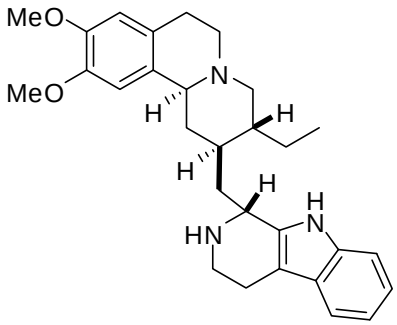
used in traditional Chinese medicine. Pharmacological evaluation revealed that it inhibits the growth of HL-60 cell in vitro by 63% at 10 μM concentration.



Chrysotricine (**9**)

1.2.1.3 Deoxytubulosin:

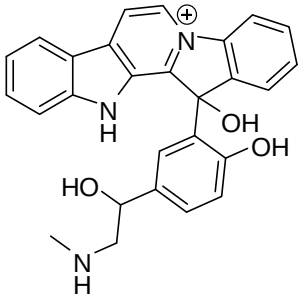
Dihydrofolate reductase (DHFR) is considered as a very attractive target for development of anticancer drugs. Biochemical and biological evaluation of β-carboline-quinolinozidine alkaloid, deoxytubulosin (**10**),[21] isolated from flower of Indian medicinal plant *Alangium lamarckii*,[22] against DHFR isolated from vitamin B12-supplemented cell of *Lactobacillus leichmannii* as the probe enzyme, resulted in identification of its DHFR inhibitory potential with IC_{50} value of 30μM. It has bacteriostatic effect on *L. Leichmannii* cells in synergy with DHFR inhibition indicates its high potential as anticancer and antibacterial agent.



Deoxytubulosin (**10**)

1.2.1.4 Thorectandramine:

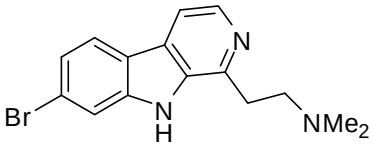
Thorectandramine (**11**), a hexacyclic quaternary β-carbolinium alkaloid was isolated from a cytotoxic extract of Paulan sponge *Thorectandra* sp. It has weak cytotoxicity against four human tumor cell lines, MALME-3M (melanoma), MCF-7 (breast), OVCAR-3 (ovarian) and A549 (non-small lung cell cancer) with EC_{50} value ranging from 27.0–55.0 μg/mL. [23]



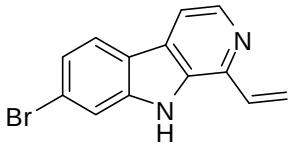
Thorectandramine (**11**)

1.2.1.5 Plakortamines:

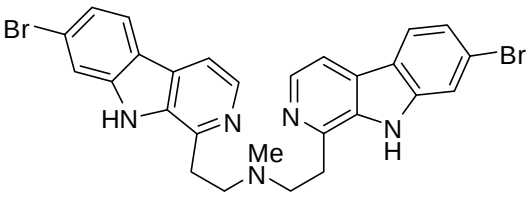
Bioassay guided fractionation of aqueous extract of deep water dark brown sponge *Plakortis nigra* collected in Palau resulted in isolation of four new cytotoxic β-carboline alkaloids plakortamines A–D (**12-15**).[24] All the four alkaloids were tested against human colon cancer cell line (HCT-116) and were found cytotoxic to these cells with IC_{50} values of 3.2, 0.62, 2.15 and 15 μM, respectively.



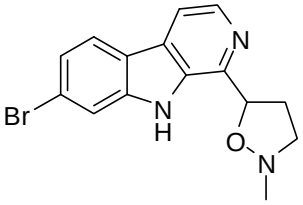
Plakortamine A (**12**)



Plakortamine B (**13**)



Plakortamine C (**14**)



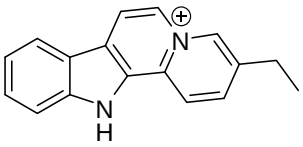
Plakortamine D (**15**)

1.2.1.6 Flavopereirine:

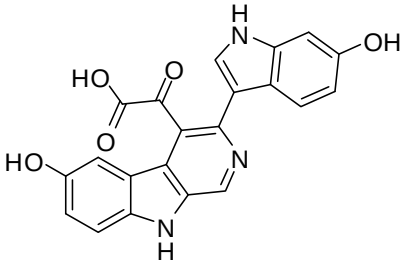
β-carboline alkaloid flavopereirine (**16**) was isolated from the bark of Amazon tree *Geissospermum sericeum* (Sagot) by Simmond's group in 2002.[25] Earlier it has also been isolated from bark of *G.laeve*, *Strychnos longicaudata*, and *S. melinoniana.*[26] It showed IC_{50} value of 10.7 ± 1.45 μM against KB (oral cancer) cell line.[27] Flavopereirine (**16**) has antiplasmodial activity against K1 and T9-96 strains of *P. falciparum* with IC_{50} values of 11.53 and 1.83 μM, respectively.

1.2.1.7 Hyrtioerectines:

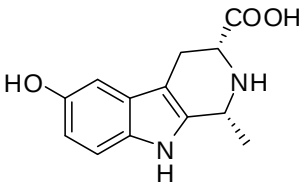
Hyrtioerectines A (**17**) and B (**18**) were isolated from ethyl acetate fraction of MeOH/DCM extract of the Red Sea sponge *Hyrtios erectus*. Both were found moderately cytotoxic against HeLa cells with IC_{50} values of 10 and 5 μg/mL, respectively.[28]



Flavopereirine (**16**)



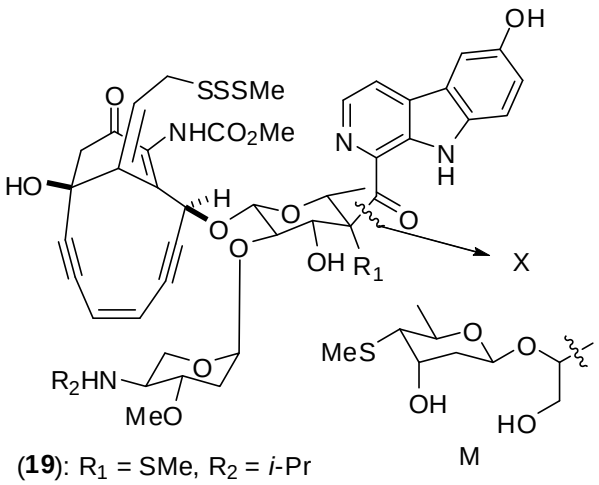
Hyrtioerectine A (**17**)



Hyrtioerectine B (**18**)

1.2.1.8 Shishijimicins A – C:

Investigations of the lipophilic extracts of the thin encrusting orange ascidian *Didemnum proliferum* collected in South Japan, resulted in isolation of three new β-carboline compounds shishijimicins A–C (**19-21**) and one known compound namenamicin (**22**) belonging to enediyne family of antibiotics. All the three shishijimicins have shown good cytotoxicy in range of 0.47-4.8 pg/mL as listed in Table 1. These compounds have unique mode of action and great promise to cancer chemotherapy. [29]



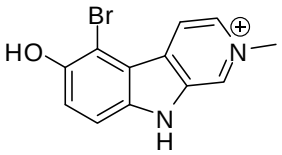
(**19**): R_1 = SMe, R_2 = *i*-Pr
(**20**). R_1 = H, R_2 = *i*-Pr
(**21**). R_1 = SMe, R_2 =Et
(**22**). R_1 = SMe, R_2 = *i*-Pr, X = M

Table 1. Cytotoxicity of (**19-22**).

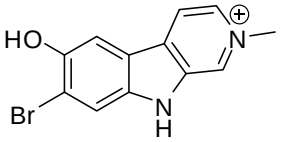
Compound No.	Cytotoxicity (IC_{50}, pg/mL)		
	3Y1	HeLa	P388
19	2.0	1.8	0.47
20	3.1	3.3	2.0
21	4.8	6.3	1.7
22	13	34	3.3

1.2.1.9 Eudistomins:

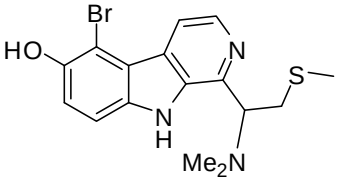
Bioassay guided fractionation of cytotoxic organic extract of marine ascidian *Eudistoma gilboverde*, yielded three new eudistomins named as 2-methyleudistomin D (**23**), 2-methyleudistomin J (**24**), 14-methyleudistomidin C (**25**).[30] Of these three new compounds, 14-methyleudistomidin C (**25**) exhibited the most potent cytotoxic activity as listed in Table 2.



2-Methyleudistomin D (**23**)



2-Methyleudistomin J (**24**)



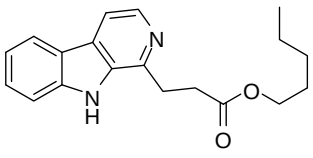
14-Methyleudistomidin C (**25**)

Table 2. Cytotoxic activities of compounds (**23-25**)

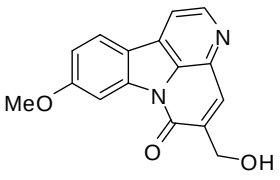
Compound No.	Cytotoxicity (μg/mL)			
	LOX (melanoma)	OVCAR-3 (ovarian)	COLO-205 (colon)	MOLT-4 (leukemia)
23	15.0	20.0	19.1	16.6
24	15.1	20.0	15.1	17.5
25	0.41	0.98	0.42	0.57

1.2.1.10 Canthinones:

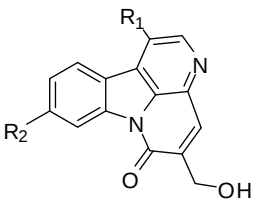
In their efforts towards search for naturally occurring medicinal agents, Wu and co-workers isolated five β-carboline alkaloids, *n*-pentyl β-carboline-1-propionate (**26**), 5-hydroxymethyl-9-methoxycanthin-6-one (**27**), 1-hydroxy-9-methoxycanthin-6-one (**28**), 9-methoxycanthin-6-one (**29**) and canthin-6-one (**30**) from the chloroform



n-Pentyl β-carboline -1-propionate (**26**)



5-Hydroxymethyl-9-methoxy canthin-6-one (**27**)



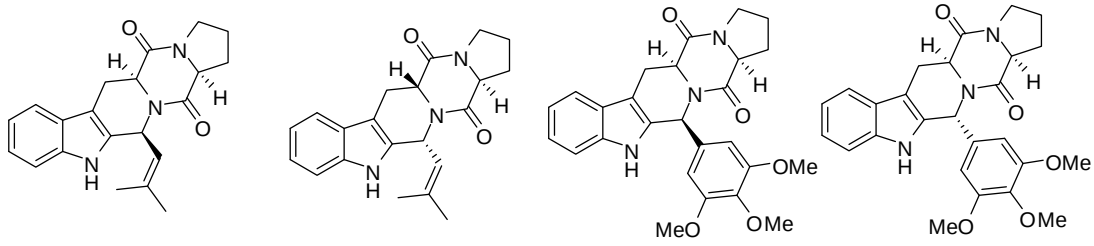
1-Hydroxy-9-methoxycanthin-6-one (**28**): R_1 = OH, R_2 = OMe
9-Methoxycanthin-6-one (**29**): R_1 = H, R_2 = OMe
Canthin-6-one (**30**): R_1 = H, R_2 = H

extract of methanol fraction of roots of Malaysian plant *Eurycoma longifolia*. Compounds **29** and **30** were identified as strongly cytotoxic toward A549 (lung cancer) cells with ED_{50} values of < 2.5 and 3.6 µg/mL, respectively.[31]

1.2.2 Synthetic β-carbolines:

1.2.2.1 Demethoxyfumitremorgin analogues:

Demethoxyfumitremorgin C (**31**), a prenylated diketopiperazine alkaloid that arrests cell cycle at G2/M phase, was isolated from fermentation broth of fungus *Aspergillus fumigatus*.[32] Ganesan et al. synthesized a few analogues of demethoxyfumitremorgin C for SAR. They found that unnatural trans epimer of demethoxyfumitremorgin C (**32**) was inactive but stereochemistry doesn't have much effect on the phenyl analogues **33** and **34** as both have same activity.[33]



Demethoxyfumitremorgin C (**31**) (**32**) (**33**) (**34**)

1.2.2.2 Indolizino[8,7-*b*]indoles:

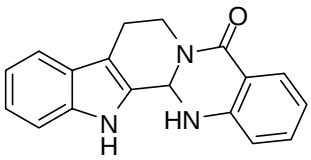
Taking inspiration from previous reports on smaller ring analogues of natural products, Dodd and co-workers designed hexahydroindolizino[8,7-b]indole derivatives which were than obtained by 1,3-Dipolar cycloaddition of acrylonitrile to 3,4-dihydro-β-carboline ylides.[34] Compound **35** and **36** showed antiproliferative activity again L1210 tumor cells with IC_{50} values of 15.6 and 16.9 µM, respectively. Both were found to arrest cycle in G2/M phase at 25 µM concentration which point toward the interaction

(**35**) (**36**)

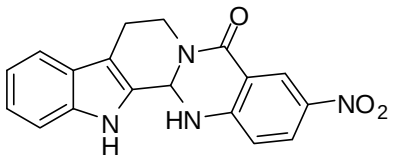
of these compounds with specific cellular components. Both of these compounds have also been found active against doxorubicin resistant as well as sensitive cells with IC_{50} values in range of 8-9 μM.

1.2.2.3 Rutaecarpine analogues:

Rutaecarpine (**37**), a β-carboline alkaloid was isolated from the fruits of plant *Evodia rutaecarpa*,[35] has interesting medicinal properties but anticancer activity was studied by Yeleswarapu et al. They synthesized a number of E ring modified analogues of rutaecarpine and found compound **38** as potent cytotoxic agent with GI_{50} values in the range of 2-3 μM against a number of cancer cell lines but did not translate the activity in to tumor xenograft model.[36]



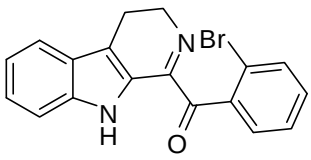
Rutaecarpine (**37**)



(**38**)

1.2.2.4 Fascaplysin analogues:

Use of fascaplysin (**8**) as anticancer drug was limited due to high toxicity, which may arise due to its intercalation in to DNA leading to toxicity. Fascaplysin is also a specific and good inhibitor of CDK-4 cyclin D-1 and inhibits the cell cycle in both cancer and normal cell at G2/M phase.[37] Inspired by this, Jenkin et al. designed and synthesized nonplanar analogues of fascaplysin. They found compound **39** as good inhibitor of CDK-4 cyclin D1.[38]

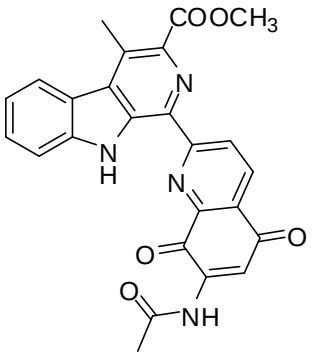


(**39**)

1.2.2.5 Lavendamycins:

Behforouz and his colleagues synthesized Lavendamycin analogues for screening against ras modified cancer cells. They discovered lavendamycin derivatives **40** as

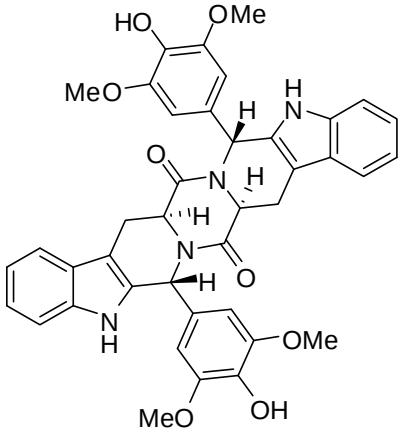
good antitumor agent having high activity against K-ras transformed cancer cells. It showed in vitro IC_{50} values of 9.0, 1.3 and 0.3 μM against K/1, K-ras transformed normal rat kidney epithelial; H/1.2, H-ras transformed normal rat kidney epithelial; N/4.2, N-ras transformed normal rat kidney epithelial; and 3LL, Lewis Lung carcinoma, respectively. Compound **40** showed 69 ± 5% inhibition on day 10 in animal treated with 100 mg/kg/day dose for 7 days.[39]



(**40**)

1.2.2.6 β-Carboline homodimers:

Deveau and co-workers[40] serendipitously discovered that β-carboline homodimers containing (a) seven consecutive fused rings (b) central diketopiperazine core (c)



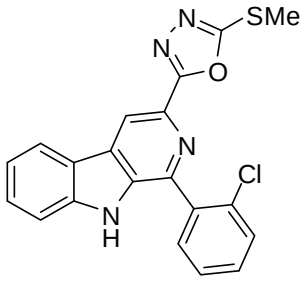
(**41**)

indole ring caping each end, and having structural similarities with elipticine, azatoxin, gypsetin, tryprostatin as good cytotoxic agents. They synthesized three pairs of homodimer enantiomers and screened them for their cytotoxic activity. They

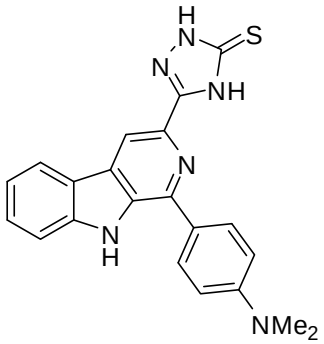
found compound **41** with IC_{50} values of (21.5 ± 1.1) and (21.9 ± 3.1) μM against lung (NCI H-520) and Prostate (PC-3) cancer cell lines, respectively as the best molecule.

1.2.2.7 3-Heteroary substituted β-carbolines:

Excited by the antitumor activity of β-carbolines and several compounds containing 1,3,4-oxadiazole, 1,2,4-triazole units, Saraggioto's group synthesized a series of 1-(substituted phenyl)-β-carbolines bearing the 2-substituted 1,3,4-oxadiazol-5-yl and 5-substituted 1,2,4-triazolyl-3-yl at C-3 expecting to improve the cytotoxicity and selectivity of β-carbolines. They identified compound **42** as good cytotoxic agent with IC_{50} value of 0.01 μM against ovarian OVCAR and 0.17 μM against Leukemia (K-562) cells lines, respectively. Triazole derivative **43** also showed good activity with IC_{50} values of 0.04 and 0.06 μM against Lung (NCI-460) and Renal (786-O) cancer cell lines, respectively.[41]



(**42**)

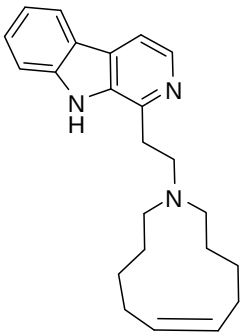


(**43**)

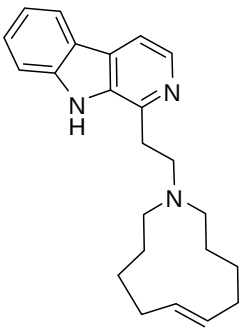
1.2.2.8 Manzamine C congeners:

Nakagawa et al. synthesized modified azacyclic ring congeners of manzamine C (**44**) i.e. one with trans geometry at double bond of azacycloundecene ring **45** and other was saturated analogue **46**.[42] Comparison of cytotoxicity of (**44–46**) against various tumor cell lines revealed that double bond in the azacycloundecene ring doesn't affect activity as all the three isomers cis, trans and saturated analogue have almost same activity. Biological evaluation of smaller ring analogues against P388, P388/MDR demonstrated that 11 membered azacycloundecene ring was essential for the activity. Coldham and co-workers[43] synthesized a series of simple analogues of Manzamine C for various biological tests. Among them, compound **47** exhibited moderate inhibition of cancer cell proliferation with IC_{50} values of 3.2, 0.38, 2.7, 2.0, 2.3, 1.6

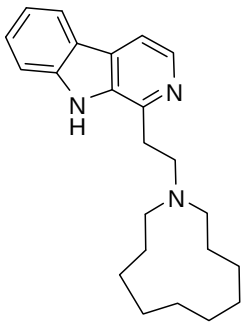
and 5.2 μM against leukaemia (K-562), non small cell lung cancer (HOP-92), colon (HT29), melanoma (M14), melanoma (UACC-62), ovarian (OVCAR-3) and breast cancer cell lines (MDA-MB-231), respectively.



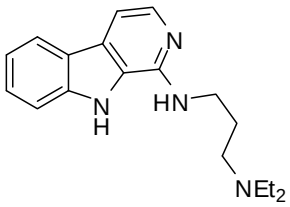
Manzamine C (**44**)



(**45**)



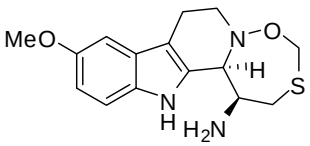
(**46**)



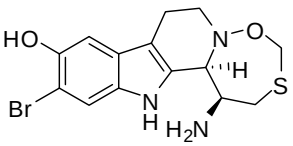
(**47**)

1.2.2.9 Eudistomin derivatives:

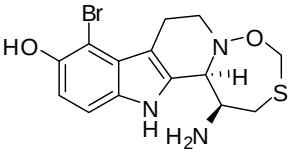
Kruse et al. discovered 10-methoxydebromoeudistomin K (**48**) as a good anticancer agent while developing an SAR of eudistomins for antiviral activity. Compound **48** is 10-30 folds more active than most potent naturally occurring eudistomin C (**49**) and E (**50**). Seven membered D ring with cis configuration at two chiral centres and an amino group at C-1 were essential for activity. Anticancer potency increase upon introduction of bromine, hydroxyl, methoxy substituents at 9, 10 and 11 positions. Activity was lost upon opening of the D ring of the eudistomins.[44]



10-Methoxydebromoeudistomin K (**48**)



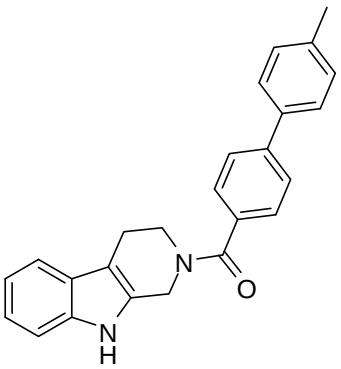
Eudistomin C (**49**)



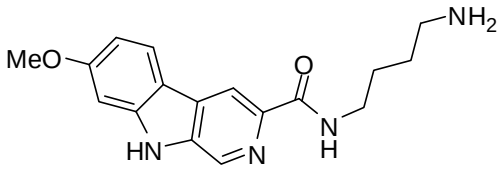
Eudistomin E (**50**)

1.2.2.10 Fascaplysin derivatives:

Taking inspiration from cyclin dependant kinase (CDK-4) inhibitory potential of fascaplysin, a new series of tetrahydro-β-carboline biphenyl derivatives was designed and synthesized.[45] Interestingly, screening of those compounds against CDK-4 provided compound **51** which inhibits cyclin dependant kinase with IC_{50} value of 9 μM. Compound **51** having para biphenyl plus para substituents supported the existence of π stacking pocket within the active site of CDK-4 enzyme.



(**51**)

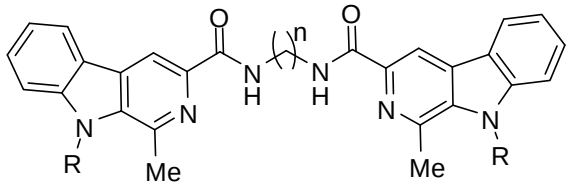


(**52**)

1.2.2.11 β-Carboline-3-carboxamides:

To study the effect on the DNA intercalating abilities of the β-carbolines, flexible alkylamine side chain analogues have been synthesized and studied for their antitumor activities. Compound **52** exhibited strongest stabilization of CT-DNA with ΔT_m = 5.7 °C and high inhibition rate for HL-60 and BGC cells with IC_{50} values of 1.9 and 8.6 μM, respectively.[46]

Xu et al. synthesized a series of 41 β-carboline derivatives[47] and screened them for cytotoxicity against human cancer and normal cell lines in an attempt to discover antiproliferative agent which are selectively cytotoxic towards cancer cell lines. It was proved that DNA targeting side chain as R_2 ($CONHCH_2CH_2NH_2$) in position 3 is responsible for H-bonding with DNA base pairs and strong intercalation in DNA.



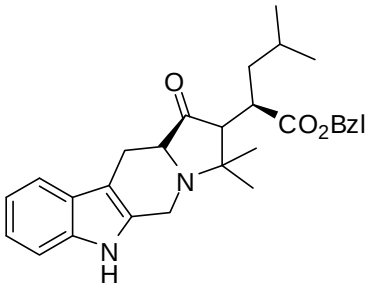
(**53**) n = 6, R = C_2H_5
(**54**) n = 2, R = $CH_2C_6H_5$

However, amidation of the DNA intercalating side chain could endow the compound to selectivity against cancer cells than to normal cells due to decrease in ability to form hydrogen bonds and decreased intercalation ability that will in turn decreases the cytotoxicity against normal cells. Due to more activity of cancer cells, their amidases should be able to cleave the amide bond much faster than normal cells, hence this should lead to increased cytotoxicity against cancer cells. Bulky groups in position 9

were not able to decrease in cytotoxicity against normal cells indicated that cytotoxicity to normal cells is not due to intercalation in DNA only, it also work via some other mechanism. β-Carboline dimers **53** and **54** were found to be most active and have IC_{50} values against VSMC (normal cell line) 7.5 and 11.6 times higher than that to C6 (tumor cell line), respectively.

1.2.2.12 β-Carboline amino acid ester conjugates:

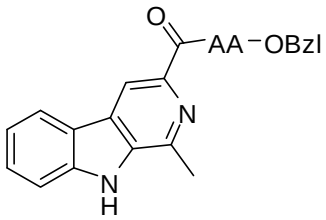
Discovery of fumitremorgin C (FTC) (**31**), a β-carboline alkaloid, as selective inhibitor of breast cancer resistance protein (BCRP/ABCG2) inspired Peng's group to synthesize some dual acting molecules possessing both resistance reversal and anticancer properties.[48] Taking advantage of their previous experience of SAR of β-carbolines and above mentioned finding, they designed the molecules having structural combinations of FTC, imidazoline, β-carboline aminoacid benzyl ester and synthesized a series of fourteen 2-substituted tetracyclic derivative of β-carbolines. They discovered compound **55** as dual acting agent which sensitized cancer cells towards doxorubicin and also showed 38.9% growth inhibition of doxorubicin resistant cells MES-SA/Dx5 cells at 1.0 μM concentration. Treatment of MES-SA/Dx5 cells with 1.0 μM concentration of compound decreased the IC_{50} value of doxorubicin against these cells from 1.53 ± 0.32 to 0.33 ± 0.05 μM.



(**55**)

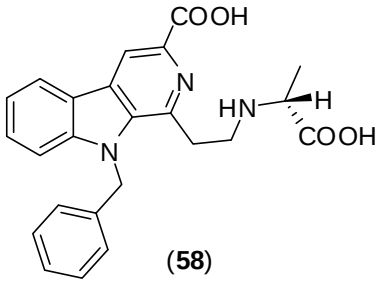
Keeping in view the fact that amino acids with functional side chains are capable of making base specific contacts with more than one type of DNA bases, Peng et al. synthesized a series of β-carboline amino acid ester conjugate to enhance the cytotoxic potency and bioavailability of β-carbolines. They found that compounds having AA = lysine (**56**) and arginine (**57**) were most active with IC_{50} values ranging from 1-7 μM against HeLa, MCF-7, HePG2 cell lines.[49] High activity of these two

derivatives were attributed due to flexible side chains of Lysine and arginine, their potential to form ionic bonds with phosphate back bone, and to donate multiple hydrogen bonds leading to strong intercalation to DNA of rapidly dividing cells.



(**56**) AA = Lysine
(**57**) AA = arginine

Peng and co-workers[50] designed a series of 16 conjugate compounds based on β-carboline-3-carboxylic acid, amino acid conjugate at C-1, benzyl moieties at N-9 for studying their anticancer activities. In vitro cytotoxicity screening led them to identification of conjugate **58** with IC_{50} values of (28.1 ± 7.5), (122.0 ± 11.0), (81.8 ± 8.3), (242.0 ± 6.9) and (120.0 ± 6.5) μM against HL-60, Hela, H1299, HepG2 and MES-SA cell lines, respectively. Compound **58** also exhibited 55.2 ± 6.7% inhibition of tumor growth in S 180 mice model at the dose of 89 μmol/kg.



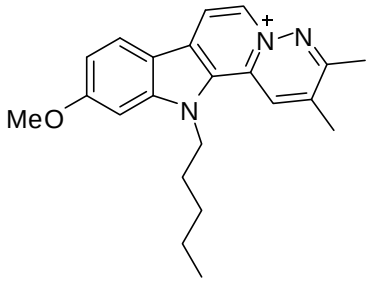
(**58**)

By docking studies using the Ligand-Fit/Ligand-Score in Discovery Studio (DS) Modeling 2.1, d(CGATCG)2 oligonucleotide retrieved from the Protein Data Bank (1D12), as the interaction model, it was confirmed that amino acid part make at least six hydrogen bonds with DNA base pairs which makes it good DNA intercalating agent.

1.2.2.13 Annulated β-carbolines:

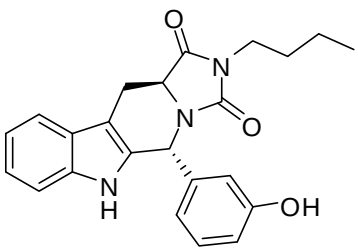
Alvarez-Builla et al. synthesized several pyridazino[1',6':1,2]pyrido[3,4-*b*]indol-5-inium derivatives starting from commercially available β-carbolines harmane,

harmine, harmol, with variations in the substituent at the phenyl ring, at *N*-9 of β-carboline nucleus and at pyridazine nucleus for improving DNA intercalating abilities. From SAR it was evident that 5-carbon alkyl chain at *N*-9 of β-carboline nucleus and methyl substituent at pyridazine ring enhances the cytotoxic activity of this series of compounds.[51] They identified compound **59** as most potent with IC_{50} value of 48 nM against L1210 leukemia cells.

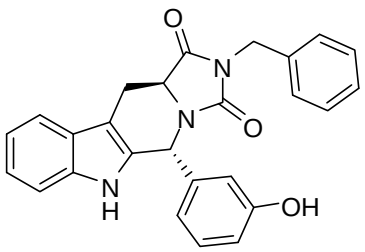


(**59**)

Screening a compound library of 16,000 small molecules against the mitotic kinesin Eg5 resulted in identification of a small molecule HR22C16 (**60**), which was found tp induce mitotic arrest and kill taxol resistance cells also. Based on this, Giannis group[52] synthesized a library of 60 related compound and discovered compound **61** as specific and potent inhibitor of mitotic kinesin Eg5 with IC_{50} value of 0.65 μM.



HR22C16 (**60**)

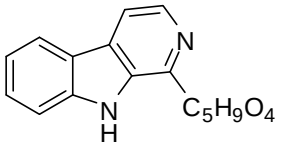


(**61**)

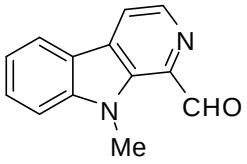
Trans isomers with absolute stereochemistry at carbon C-11 being S-configured were active while corresponding cis isomers were unable to inhibit the kinesin Eg5-ATPase activity. m-Hydroxyphenyl ring at C-1 of the tetrahydro-β-carboline ring system was also found to be important for activity.

1.2.2.14 Simple 1-substituted β-carboline derivatives:

Anticancer screening of all the intermediates involved in the synthesis of a diastereomer of compound **62** by El-Shorbagi and co-workers led them to identification of antiproliferative activity of β-carboline aldehyde **63**.[53] It has CC_{50} value of 0.36 μM against human promyelocytic leukemia cells HL60.

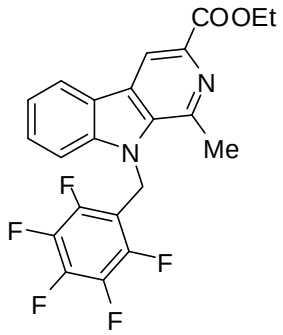


(**62**)



(**63**)

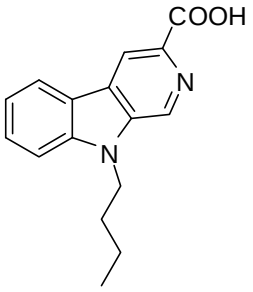
Xu group elucidated the preliminary structure activity relationship of β-carboline derivative by simple structural modifications and probing structural requirement of these compounds for potent antitumor activity. They synthesized a series of 1,3-disubstituted and 1,3,9-trisubstituted β-carbolines and evaluated them for cytotoxicity against a panel of human cancer cell lines. It was found that trisubstiuted analogues were more active than their disubstituted analogues.[54] Among all the synthesized compounds, trisubstituted β-carboline **64** has best antitumor activity in vitro with IC_{50} value of 4 μM against BCG-823 (gastric carcinoma) cell line.



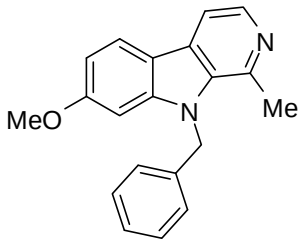
(**64**)

To study the effect of various alkyl group at *N*-9 of β-carboline core on the toxicity, Xu's group synthesized a number of *N*-9 alkyl substituted derivative of harmine and β-carbolines obtained from L-tryptophan. After cytotoxicity evaluation of these synthesized analogues they concluded that alkyl or benzyl substituents at 9-position of the β-carboline ring and alkoxycarbonyl at 3-position plays important roles in decreasing acute neurotoxicity and increase in antitumor activity. Compound **65** reduced the tumor volume by 46.9 and 43.1% in the mice bearing Lewis lung carcinoma and Sarcoma 180, respectively.[55] Compound **66** showed LD_{50} value of >

500 mg/kg at 95% C.L. in mice with no neurotoxic effect but compound **66** was acutely neurotoxic at the dose of 26.45 mg/kg at 95% C.L. in mice.

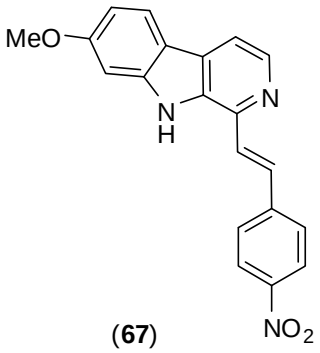


(**65**)



(**66**)

Inspired by good cytotoxicity of chloroform extract of the Chinese plant *Symplocos serchensis*, Lee and co-workers[56] synthesized a few β-carboline analogues evaluated them for cytotoxicity against a panel of human cancer cell lines. They discovered that introduction of alkoxy substituent at C-7 of β-carboline ring increase the activity, while length of the alkoxy chain was also found to be important for cytotoxicity and cell specificity. *N*-9 alkylation leads to increase in cytotoxic effects against KB and

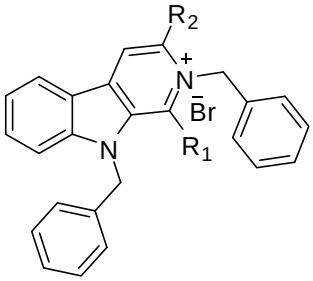


(**67**)

IA9 cells. Compound **67** having 4-nitrobenzylidene substitution at C-1 was most active with ED_{50} values of 0.3 to 1.2 μg/mL against epiderimoid carcinoma of the nasopharynx (KB), lung carcinoma (A-549), ileocecal carcinoma (HCT-8), renal cancer (CAKI-1), breast cancer (MCF-7), melanoma cancer (SK-MEL-2), ovarian cancer (IA9), glioblastoma (U-87-MG), osteosarcoma (SaOS-2), osteosarcoma (HOS), and embryonic lung fibroblast (HEL)..

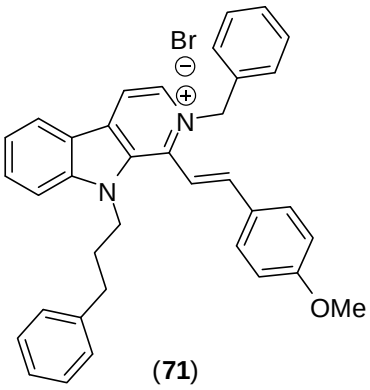
In continuation of their previous efforts and on the basis of harmine (**3**) chemical structure, Xu's group designed and synthesized differently substituted β-carbolines in order to improve the efficacy and decrease in toxicity as compared to harmine. They

identified the three derivatives **68**, **69** and **70** being most potent with IC_{50} values either comparable to harmine or less than harmine against several cancer cell lines. From SAR it was also evident that a benzyl group at both position 2 and 9 increase antitumor activity as well as acute cytotoxicity.[57]



(**68**): R_1 = Me, R_2 = H
(**69**): R_1 = H, R_2 = COOEt
(**70**): R_1 = H, R_2 = H

In their continuous efforts towards synthesis of novel β-carbolines as efficacious antitumor agents with lower side effects, Peng et al. designed a new series of β-carboline derivatives having benzylidine substituent at 1-position of β-carboline ring.

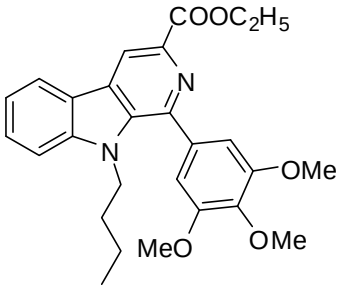


(**71**)

Anticancer screening of the synthesized analogues led them to identification of compound **71** as most potent antitumor agent with IC_{50} values ranging from 0.93 to 21 μM against various cancer cell lines.[58]

To study the SAR and probe the structural requirement for the potent antitumor activity of β-carbolines, a series of variously substituted tetrahydro-β-carbolines were synthesized starting from L-tryptophan and 3,4,5-trimethoxybenzaldehyde. Antitumor screening of which resulted in establishment of fact that (a) a small alkyl chain at the position 9 of the β-carboline ring plays an important role in cytotoxic

activities, (b) differently substituted β-carbolines interact with different targets leading to different antitumor activities.[59] Compound **72** was most active with IC_{50} value of 27.3 and 43.3 μM against BCG-823 (gastric carcinoma) and MCF-7 (breast) cancer cell lines, respectively.



(**72**)

1.3 Antileishmanial β-carbolines:

1.3.1 Harman derivatives:

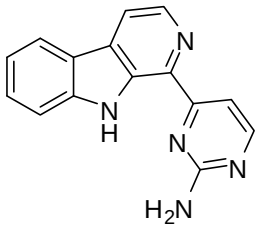
Inspired by wide range of pharmacological activities shown by β-carboline alkaloid isolated from plants, Di Giorgio and co-workers investigated harman (**1**), harmine (**3**) and harmaline (**4**) for their antileishmanial activity against *Leishmania infantum*. Harmaline was found to be most potent of the three alkaloids with IC_{50} value of 1.16, 116.8 μM against amastigotes and promastigotes stages, respectively and more importantly selectivity index of >170.[60] Although harman and harmine had shown lower IC_{50} values but their selectivity indices were poor. Harmaline acts via unique mechanism of action based on inhibition of leishmania protein kinase C(PKC) activity in leishmania promastigotes leading to a reduction of parasite internalization within macrophages.

1.3.2 Annomontine:

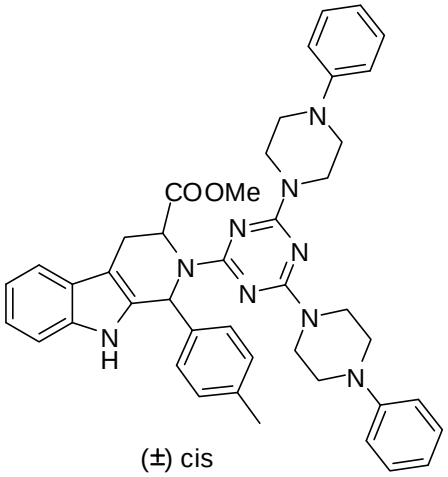
In the search of novel antileishmanial agents, Pinheiro's group investigated the bark of *Annona foetida*, a tropical native tree found in Brazilian and Peruvian Amazon, and isolated a pyrimidine-β-carboline alkaloid, annomontine (**73**) by bioassay guided fractionation.[61] Annomontine showed IC_{50} value of 34.8 ± 1.5 μM against *Leishmania brazilensis* (promastigotes forms).

1.3.3 Triazinotetrahydro-β-carboline analogues:

At the start of our project on synthesis of antileishmanial agents, our group has synthesized some hybrid molecules having 2,4,6-triamino-1,3,5-triazine heterocycles along with isoquinoline and tetrahydro-β-carboline groups as possible antileishmanial agents. We discovered compound **74** as good antileishmanial agent showing 78.6% *in vivo* inhibition at a dose of 50 mg/kg ×5days.[62]



Annomontine (**73**)



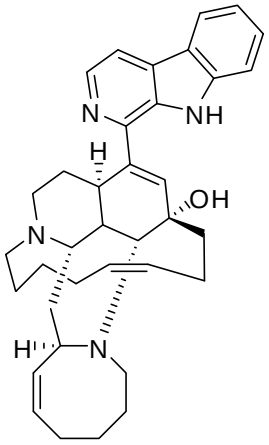
(±) cis

(**74**)

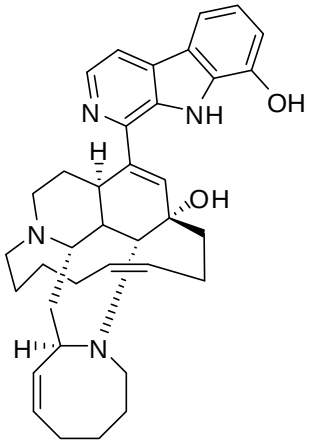
1.4 Antimalarial β-carbolines:

1.4.1 Manzamines:

Manzamine A (**75**), a complex and unique β-carboline alkaloid was initially isolated from Haliclona sp. but has subsequently been isolated from various species of marine sponges found in waters of Indian Oceans and Pacific Oceans.[63-65] Both manzamine A and 8-hydroxymanzamine (**76**) were tested *in vivo* in mice infected with the erythrocytic stage of *Plasmodium berghei*. It was found that both the compounds are active against asexual erythrocytic stage of *Plasmodium berghei.* Manzamine A is also active against chloroquine and mefloquine resistant parasite. Despite having low therapeutic value, mice treated with manzamine A remain alive for a longer time period than chloroquine.[66]

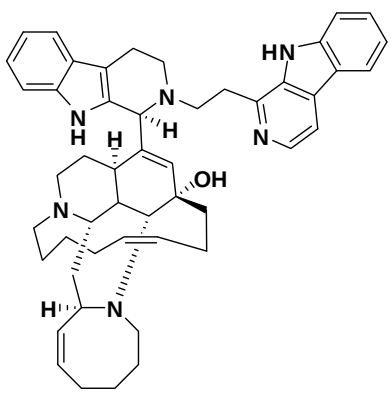


Manzamine A (**75**)

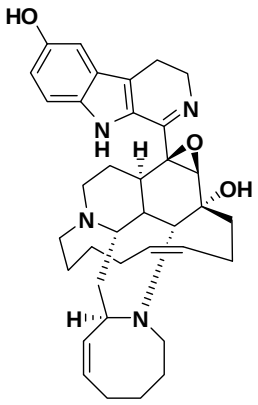


8-Hydroxymanzamine A (**76**)

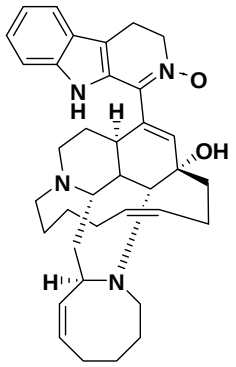
Three manzamine alkaloids zamamidine C (**77**), 3,4-dihydro-6-hydroxy-10,11-epoxymanzamine A (**78**), and 3,4-dihydromanzamine J N-oxide (**79**) having unique polycyclic structures were isolated from Okinawan marine sponge of the genus Amphimedon by Kobayashi and co-workers.[67] Being aware of the fact that manzamine alkaloids have been reported to have many biological activities, they screened all three against *Plasmodium falciparum, Trypanosma brucei brucei* and three human cancer cell lines P388, L1210 (murine leukemia) and KB (human epidermoid carcinoma). Biological activity data is tabulated in Table 3. Zamamidine C (**77**) was most active against *P. falciparum* with IC_{50} value of 0.58 μg/mL while 3,4-dihydromanzamine J N-oxide (**79**) was most active against *T. brueci brueci* with IC_{50} value of 0.04 μg/mL.



Zamamidine C (**77**)



3,4-Dihydro-6-hydroxy-10,11-epoxymanzamine A (**78**)

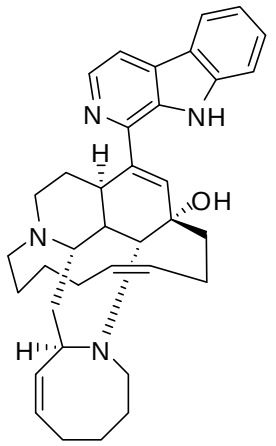


3,4-Dihydromanzamine J N-oxide (**79**),

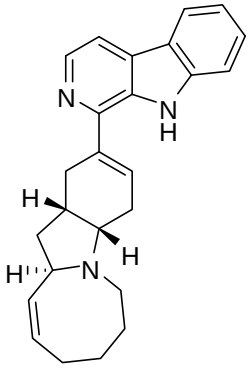
Table 3. Biological activity data of compounds (**77**, **78**, **79**)

Compound No.	Biological activity in vitro (IC_{50}, µg/mL)				
	P. falciparum	*T. brucei brucei*	P388 (murine leukemia)	L1210 (murine leukemia)	KB (human epidermoid carcinoma)
77	0.58	0.27	14.1	13.6	17.7
78	7.03	0.44	3.6	3.9	6.2
79	0.97	0.04	3.6	9.0	6.7

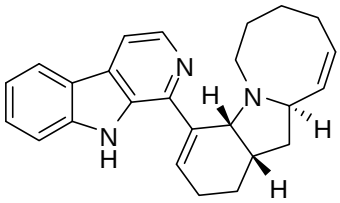
Low isolated yield and idea of investigating the role of A and D rings, effect of change in relative stereochemistry and orientation of β-carboline core on antimalarial activity inspired Winkler's and Hamann's group to design and synthesize simplified analogues of manzamine A. After initial screening it was found that compounds **80** and **81** having same relative stereochemistry and orientation of β-carboline core were active against malarial parasite in vitro but were significantly less active than manzamine A (**75**) itself.[68]



Manzamine A (**75**)



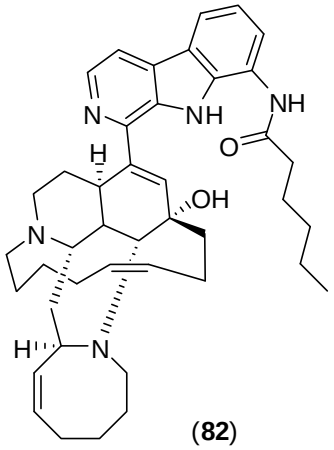
(**80**)



(**81**)

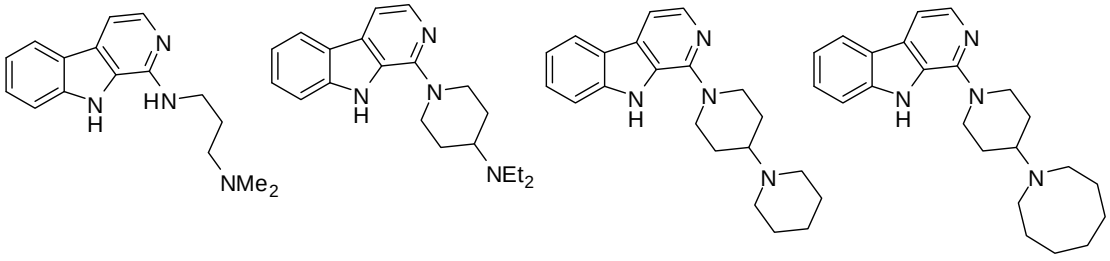
To reduce the intercalating ability into DNA, hence the toxicity, it was thought that introduction of a bulky alkyl chain of manzamine A (**75**) which can interfere with the intercalation process might be useful strategy. In this process, amidation at C-8 of the β-carboline ring, resulted in identification of 8-hexamidoemanzamine A (**82**) analogue as potent antimalarial agent with reduced toxicity.[69] It reduced the parasitemia by

64% in the mice infected with *Plasmodium berghei* at the dose of 30 mg/kg (once daily for three days) with no apparent toxicity.



(**82**)

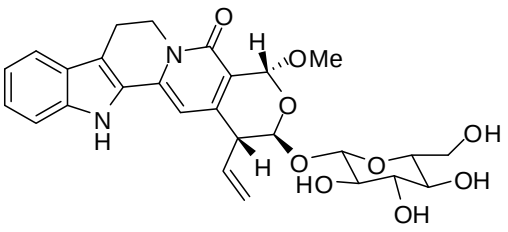
Among the simpler analogues of Manzamine C synthesized by Coldham et al. four compounds (**83–86**) showed good inhibition of *P. falciparum* with IC_{50} value ranging from 0.35 to 0.45 μg/mL, but were found to be cytotoxic.[70]



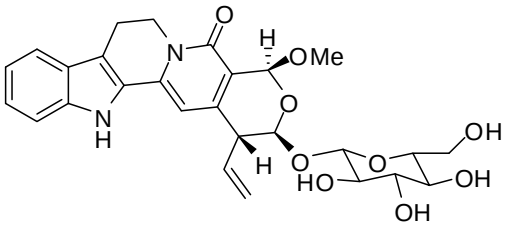
(**83**) (**84**) (**85**) (**86**)

1.4.2 Naukleaorines:

Two new tetrahydro-β-carboline alkaloids, naukleaorine and epimethoxynaukleaorine were isolated from chloroform extract of dried stem of *Nauclea orientalis* by bioassay guided fractionation. Both of the compounds were screened for their antimalarial



Naucleaorine (**87**)

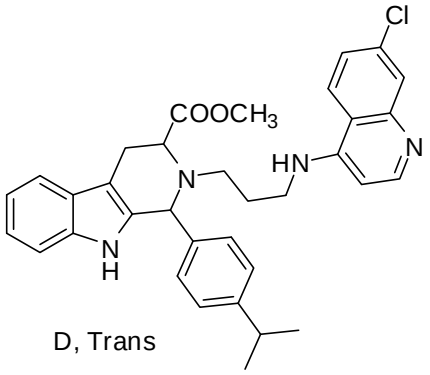


Epimethoxynaucleaorine (**88**)

activity and were found to be moderately active. Naukleaorine (**87**) showed IC_{50} and selective index values of 6.9 ± 0.6 (SI 5.5) and 8.0 ± 0.4 μM (SI 4.8); while epimethoxynaukleaorine (**88**) has corresponding values of 12.4 ± 0.3 (SI >3.1) and 13.2 ± 0.1 μM (SI >2.9) against chloroquine sensitive clone D6 and chloroquine resistant resistant clone W2, respectively.[71]

1.4.3 Quinoline-β-carboline hybrids:

With an aim to increase the potency of quinoline based drugs against resistant *P. falciparum*, we designed and synthesized 7-chloroquinoline containing dual inhibitor that would potentially inhibit haemozoin formation and also act on another target. Compound **88a** trans isomer of tetrahydro-β-carboline obtained from D-tryptophan, was most active with MIC value of 0.05 μM and was more active than chloroquine (MIC value 0.391 μM).[72]

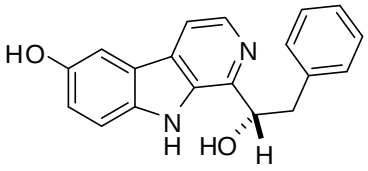


(**88a**)

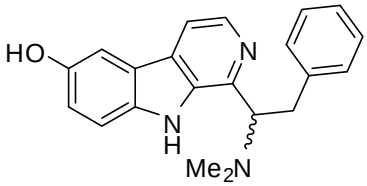
1.5 Antifungal β-carbolines:

Exhibition of strong detterent effect of MeOH extract of the Micronesian ascidian sponge of genus Eudistoma sp. towards feeding by generalist reef, led Proksch and co-workers to take up the task of chemical investigation of this extract. Bioassay guided fractionation of the extract afforded them the two UV active compounds named as eudistomin W (**89**) and eudistomin X (**90**). Both were evaluated for their antifungal and antibacterial activities and eudistomin W was found to be specific inhibitor of *Candida albicans* growth with 13mm zone of inhibition at 10 μg/disk. Similarly eudistomin W also showed 17 and 18 mm zone of inhibition at the doses of 5 and 10 μg/disk, respectively. While, eudistomin X showed zones of inhibition

against *Bacillus subtilis* (17 and 18mm), *Staphylcoccus aureus* (11 and 12 mm), *Escherichia coli* (15 and 20 mm) at dose of 5 and 10 μg/disk.[73]



Eudistomin W (**89**)



Eudistomin X (**90**)

β-carboline alkaloids harmane (**1**), harmine (**3**), harmaline (**4**), and harmalol (**6**) were also evaluated for their antifungal activity against *Candida albicans* and they exhibited MIC values of 0.1, 0.6, 0.2 and 0.4 mg/mL. However, due to their strong cytotoxicity these alkaloids cannot be considered as important antifungal leads.[74]

1.6 Conclusion:

This review gives a brief idea about β-carbolines as promising new leads for development of novel clinical drugs against cancer, malaria, leishmaniasis, and fungal diseases. Although, some of β-carbolines are highly toxic, mutagenic or precursor of mutagens but efforts should be made to exploit the pharmacological potential of other alkaloid by reducing their toxicity. Only few efforts have been made for synthesizing the analogues of β-carboline alkaloids in search of new clinical drug candidates, a lot more attention is required to obtain better leads from this class.

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Chapter 2

Design, Synthesis and Cytotoxicity Evaluation of (Tetrahydro-β-carboline)-1,3,5-Triazine Hybrids as Anticancer Agents

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2.1 Introduction:

Cancer is a major health problem worldwide and is the leading cause of human mortality exceeded only by cardiovascular diseases.[1] Therefore, development of new anticancer drugs and more effective treatment strategies for cancer are of utmost importance as traditionally prescribed chemotherapeutic agents have problems with toxicity and drug resistance.[2] Although, numerous kinase inhibitors have been discovered recently and several have been successfully developed for treatment of cancer including Gleevec,[3] Iressa,[4] Tarceva,[5] Tykerb,[6] and Sutent,[7] still there is strong demand for discovery of improved cytotoxic agents. As most of the solid human cancer tumor are multi causal in nature and their treatment with "mechanism-based" agent alone is unlikely to be successful, so a combination of these inhibitors with a better cytotoxic drug is likely to be a good strategy.[8]

The β-carboline nucleus that possess a common tricyclic pyrido[3,4-*b*]indole ring structure, is a recurring motif in both natural and synthetic cytotoxic compounds, which can act through multiple mechanisms.[9] In addition to simple, substituted harman and norharman derivatives,[10] more complex structures such as manzamine A (**1**),[11] eudistomine K (**2**),[12] azatoxin (**3**),[13] fascaplysine (**4**),[14] and picrasidine L (**5**)[15] display potent cytotoxic activities against various cancer cell lines. (Figure 1)

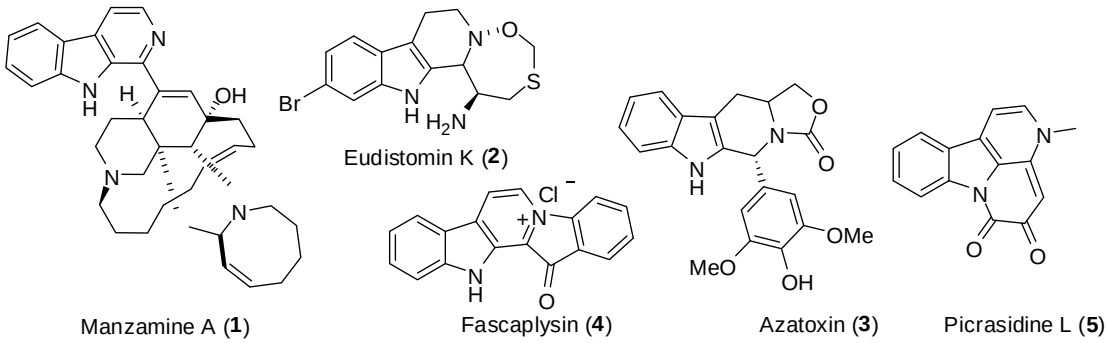


Figure 1. Cytotoxic natural products containing β-carboline nucleus.

Cytotoxicity of 1,3,5-triazine derivatives is well known as hexamethylmelamine (**6**) was discovered as an effective agent against breast, lung and ovarian cancer but it causes many adverse effects such as nausea, vomiting, anorexia and abdominal cramps.[16] Irsogladine **7** has been shown to have anti-tumor activity in murine

xenograft models of epidermoid cancer and glioma.[17] More recently, the effect of irsogladine was also investigated in a human breast cancer athymic nude mouse system and the results suggested that irsogladine can be useful in the breast cancer adjuvant setting.[18] Moon et al. reported compound **8** as a microtubule destabilizing agent with potent growth inhibition against U936 cells (GI_{50} = 1 μM).[19] p38 MAP kinase inhibitory activity[20] of **9**, inhibitory potency of **10** against various cyclin dependent kinases,[21] and VEGF-R2 (KDR) tyrosine kinase inhibitory activity[22] of **11** has also been reported recently. (Figure 2)

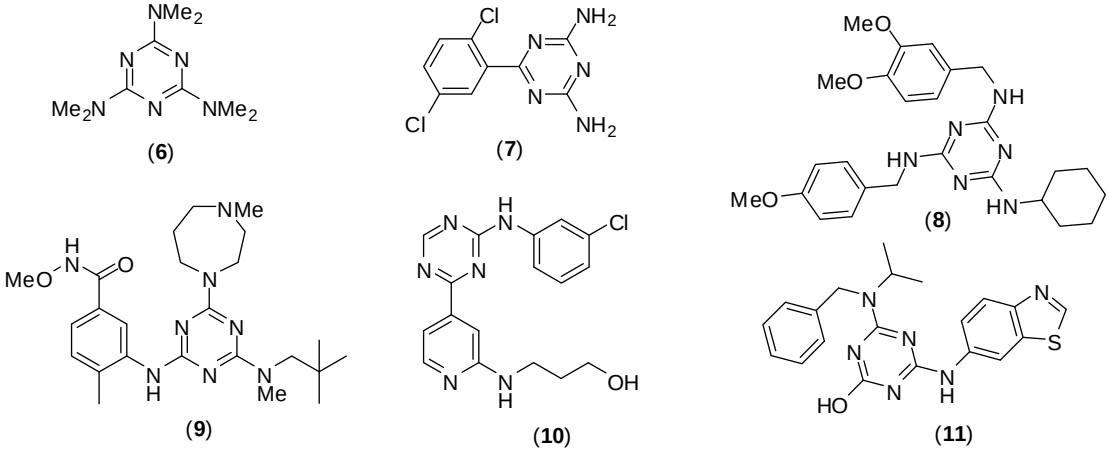


Figure 2. Previously reported anticancer 1,3,5-triazine derivatives.

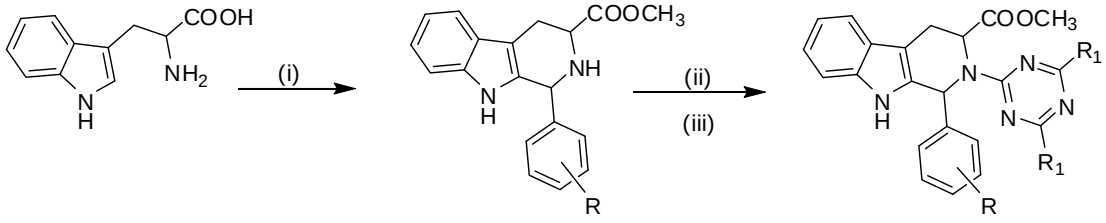
Now a days, there is an increased interest in use of hybrid molecules for drug discovery against multitude of disease indications.[23] Lesser degree of conformational flexibility and less likely interaction of tetrahydro-β-carbolines with DNA may also lead to reduced toxicity and increased anticancer activity.[24] These observations and our previous experience[25, 26] led us to hypothesize that (tetrahydro-β-carboline)-1,3,5-triazine hybrid molecules will be more efficacious and selective cytotoxic agents. Design, synthesis and cytotoxic activitiy of a series of (tetrahydro-β-carboline)-1,3,5-triazine hybrid derivatives against a panel of human cancer cell lines are described in this chapter.

2.2 Chemistry:

The synthetic strategy followed for synthesis of (tetrahydro-β-carboline)-1,3,5-triazine hybrids is depicted in scheme 1. The tetrahydro-β-carbolines (**12-28**) were

obtained via Pictet-Spengler cyclization[26] of methyl ester of tryptophan with various substituted benzaldehydes under acidic conditions. Two diastereomers so obtained were separated by flash column chromatograpy. Spectroscopic data of the obtained tetrahydro-β-carbolines (**12-28**) were identical to that of reported earlier. [24, 27]

Scheme 1. Preparation of (tetrahydro-β-carboline)-1,3,5-triazine hybrids (**29-63**)



D/L/DL-Tryptophan (**12-28**) (**29-63**)

For (**12**) R = 4-methyl, isomer = DL, Trans; (**13**) R= 4-methyl, isomer = DL, Cis; (**14**) R = 4-methyl, isomer = L, Trans; (**15**) R= 4-methyl, isomer = L, Cis; (**16**) R= 4-methyl, isomer = D, Trans; **(17)** R= 4-methyl, isomer = D, Cis; **(18)** R = 4-methoxy, isomer = DL, Trans; **(19)** R = 4-methoxy, isomer = DL, Cis; **(20)** R = 4-methoxy, isomer = L, Trans; **(21)** R= 4-methoxy, isomer = L, Cis ; **(22)** R = 4-methoxy, isomer = D, Trans; **(23)** R= 4-methoxy, isomer = D, Cis; **(24)** R = 3,4,5-trimethoxy, isomer = DL, Trans; **(25)** R = 3,4,5- trimethoxy, isomer = DL, Cis; **(26)** R = 3,4,5- trimethoxy, isomer = L, Trans; (**27**) R = 3,4,5- trimethoxy, isomer = L, Cis; **(28)** R = 4-chloro, isomer = DL, Trans.

Reagents and Conditions: (i) Thionyl chloride, MeOH, various benzaldehydes, 6h (ii) Cyanuric chloride, K_2CO_3, THF, 0 °C- rt, 2h (iii) Amines, K_2CO_3, THF, reflux, 8h.

The (tetrahydro-β-carboline)-1,3,5-triazine hybrids (**29-63**) enlisted in Table 1 were synthesized in a two step sequence[26]- (i) a nucleophilic substitution of one chloro group of cyanuric chloride with *N*-2 of different tetrahydro-β-carbolines, (ii) displacement of remaining two chloro groups with various amines. (Scheme 1) Compound **64** was also synthesized according the same procedure except that tryptamine was used instead of methyl ester of tryptophan in step (i) of above

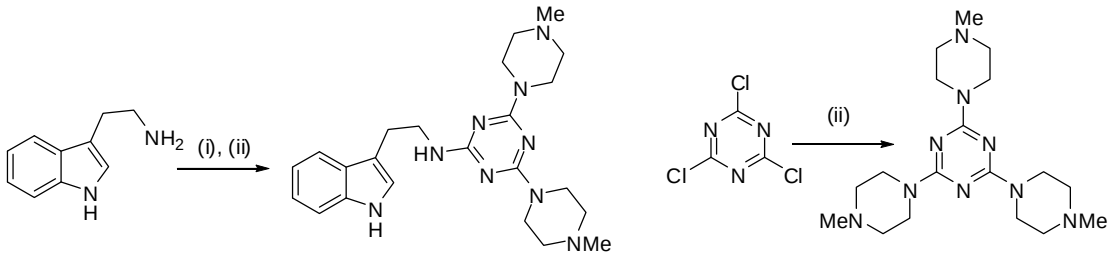
mentioned method. (Scheme 2) Treatment of cyanuric chloride with excess of *N*-methylpiperazine resulted in formation of compound **65**. (Scheme 2)

Table 1. (Tetrahydro-β-carboline)-1,3,5-triazine hybrid analogues (**29-63**)

Entry	Compound No.	Isomer	R	R_1
1	**29**	DL, Cis	4-methoxy	*N*-methylpiperazine
2	**30**	DL, Trans	4-methoxy	*N*-methylpiperazine
3	**31**	DL, Cis	4-methoxy	butylamine
4	**32**	DL, Trans	4-methoxy	butylamine
5	**33**	DL, Cis	4-methyl	2-aminoethanol
6	**34**	DL, Trans	4-methyl	2-aminoethanol
7	**35**	DL, Trans	3,4,5-trimethoxy	*N*-methylpiperazine
8	**36**	DL, Cis	3,4,5-trimethoxy	*N*-methylpiperazine
9	**37**	L, Cis	4-methoxy	*N*-methylpiperazine
10	**38**	L, Trans	4-methoxy	*N*-methylpiperazine
11	**39**	L, Cis	4-methoxy	*N*-phenylpiperazine
12	**40**	L, Trans	4-methoxy	*N*-phenylpiperazine
13	**41**	L, Cis	4-methoxy	morpholine
14	**42**	L, Trans	4-methoxy	morpholine
15	**43**	L, Trans	4-methoxy	cyclohexylamine
16	**44**	L, Cis	4-methoxy	o-toluidine
17	**45**	L, Trans	4-methoxy	o-toluidine
18	**46**	L, Trans	3,4,5-trimethoxy	*N*-methylpiperazine
19	**47**	L, Cis	3,4,5-trimethoxy	*N*-methylpiperazine
20	**48**	L, Trans	4-methoxy	*N*-ethylpiperazine
21	**49**	L, Trans	4-methoxy	*N*-propylpiperazine
22	**50**	D, Cis	4-methoxy	*N*-methylpiperazine
23	**51**	D, Trans	4-methoxy	*N*-methylpiperazine
24	**52**	D, Cis	-H	*N*-methylpiperazine

25	**53**	D, Trans	-H	*N*-methylpiperazine
26	**54**	D, Cis	4-methyl	*N*-methylpiperazine
27	**55**	D, Trans	4-methyl	*N*-methylpiperazine
28	**56**	DL, Trans	4-chloro	*N*-methylpiperazine
29	**57**	DL, Trans	4-methyl	*N*-methylpiperazine
30	**58**	DL, Cis	4-methyl	propylamine
31	**59**	DL, Trans	4-methyl	propylamine
32	**60**	DL, Trans	4-methyl	ethylamine
33	**61**	DL, Trans	4-methoxy	ethylamine
34	**62**	DL, Cis	4-methoxy	propylamine
35	**63**	DL, Trans	4-methoxy	propylamine

Scheme 2. Preparation of 2,4,6-triamino-1,3,5-triazines



(64) **(65)**

Reagents and Conditions: (i) Cyanuric chloride, K_2CO_3, THF, 0°C- r.t. (ii) *N*-methylpiperazine, K_2CO_3, THF, Reflux.

2.3 Results and Discussion:

1 to 50 μM concentrations of (tetrahydro-β-carboline)-1,3,5-triazine hybrids were tested for *in vitro* cytotoxic activity on human cancer cell lines representing breast cancer (MCF-7), colon (SW620), prostate (DU-145), oral (KB), ovary (PA1), leukemia (K562), pancreas (MiaPaCa-2), lung (A-549) and normal fibroblasts (NIH3T3) by MTT assay.[28]

Initially, anticancer activity of all the previously reported (tetrahydro-β-carboline)-1,3,5-triazine[24] hybrids by our group and synthesized analogues (**29-36**) was assessed *in vitro* against a panel of 8 human cancer cell lines and the results are summarized in Table 2. Previously reported (tetrahydro-β-carboline)-1,3,5-triazine hybrids were

found to be inactive. But we were pleased to find that compound **30** obtained from DL-tryptophan, having trans stereochemistry, 4-methoxy group as R and *N*-methylpiperazino group as R_1 showed IC_{50} value of 1.87 and 4.78 μM against KB and NIH3T3 cell lines, respectively. It was 2.5 times more selectively toxic towards KB than NIH3T3 cell lines, while its cis isomer **29** was moderately cytotoxic against all the tested cell lines. Butylamino group as R_1 as in compounds **31** and **32**, was found to be detrimental to cytotoxic activity. Also the combination of 4-methyl group as R and aminoethanol as R_1 has resulted in complete loss of activity in compounds **33** and **34.** Cytotoxicity profiles of **35** and **36** also indicated that trans isomer was preferred over cis isomer for cytotoxicity. Excited by these finding, and to rapidly develop SAR around 1,3,5-triazine moiety, enantiopure β-carboline unit having 4-methoxy group as R, obtained from methyl ester of L-tryptophan, was kept intact while R_1 was varied using various amine nucleophiles. Cytotoxic evaluation of compounds (**37-45**) demonstrated that every substituent except *N*-methylpiperazino group as R_1 had detrimental effect on cytotoxicity against human cancer cell lines. To our surprise compound **38**, which is enantiopure form of **30**, was not selective toward KB cells but was 2.6 times selectively toxic towards MCF-7 cell lines in comparison to NIH3T3 cells. On the other hand **37,** the cis isomer of **38,** was generally cytotoxic to all cell lines and has similar cytotoxicity profile as **29**. Subsequently, we tried few other combinations, hybrids (**46-49**) having R = 3,4,5-trimethoxy, 4-methyl, 4-methoxy, 3-hydroxy and R_1 = various cyclic amines, were synthesized starting from L-tryptophan and evaluated for cytotoxicity. But again, only compounds having N-methylpiperazino group as R_1 were found to be cytotoxic. Interestingly, cytotoxic activity was also found to be decreasing with increase in carbon chain length at N-4 of piperazine unit as evidenced by the cytotoxicity profile of **48** and **49**. Compound **48** with N-ethylpiperazino group as R_1 exhibited better cytotoxicity than N-propylpiperazine derivative **49**, whereas both were less active than **30**. In the meantime, compound **38** was studied for its effect on cell cycle in MCF-7 and MDA-MB-231 cell lines and found to arrest mitotic cycle in G1 phase. DNA fragmentation analysis and Hoescht staining was also carried out in MCF-7 and MDA-MB-231 cell lines to confirm apoptosis by **38**. At that time it was thought that cytotoxicity profile of other enantiomers of **37** and **38** i.e. **50** and **51** will be interesting. So, a few

analogues (**50-55**) were also synthesized starting with methyl ester of D-tryptophan. They also showed same SAR as that of compounds obtained from methyl ester of L-tryptophan, i.e. only compounds having *N*-methylpiperazino

Table 2. *In Vitro* cytotoxicity data of (tetrahydro-β-carboline)-1,3,5-triazine hybrids and 2,4,6-triamino-1,3,5-triazine derivatives

Entry	**Compound No.**	**IC_{50} (μM)**								
		MCF-7	SW620	DU-145	KB	PA1	K562	Miapaca-2	A-549	NIH3T3
1	**29**	6.06	3.32	5.35	1.46	2.72	7.13	7.37	6.30	2.33
2	**30**	12.44	4.26	10.47	1.87	2.03	NA	6.35	5.83	4.78
3	**35**	NA	3.15	2.01	2.43	2.75	3.00	7.54	5.67	2.17
4	**36**	5.96	ND	5.22	17.88	5.32	ND	ND	9.84	6.71
5	**37**	9.00	1.19	10.31	7.54	2.77	4.83	3.62	3.13	1.62
6	**38**	0.74	1.35	6.75	8.06	1.82	4.6	2.3	2.1	1.96
7	**48**	1.34	NA	1.67	1.22	NA	ND	ND	1.61	1.86
8	**49**	2.07	NA	7.11	3.54	NA	ND	ND	1.67	2.24
9	**50**	12.27	3.27	2.45	1.82	3.34	4.48	5.26	11.46	5.28
10	**51**	10.97	2.15	2.58	0.99	2.05	6.00	6.23	3.00	3.16
11	**52**	8.28	3.24	7.76	1.83	1.79	6.45	5.88	8.62	11.38
12	**53**	6.21	2.84	2.81	1.98	1.93	5.34	6.11	3.21	6.00
13	**55**	5.89	2.65	2.14	1.86	6.91	3.22	7.00	2.34	2.16
14	**56**	NA	2.73	NA	1.98	6.39	5.01	7.12	6.55	2.22
15	**57**	NA	NA	NA	0.1058	NA	NA	NA	NA	NA
16	**59**	NA	NA	NA	0.6647	NA	NA	NA	NA	NA
17	**63**	NA	NA	NA	0.1222	NA	NA	NA	NA	NA

IC_{50} = compound concentration required to inhibit tumor cell proliferation by 50%. Data are expressed as mean from the dose response curves of at least two independent experiments with three determination in each. [a]NA = not active, (IC_{50} is greater than 25 μM). ND = not done.

group as R_1 were found to be cytotoxic but they differed from **30** in their selectivity towards KB cells. A little decrease in cytotoxicity was observed when R = 4-methoxy group in **51** was replaced with H (**53**). Similar was the case with 4-methyl derivative

55, as it also showed decreased toxicity and selectivity. From the above data it was evident that racemates are more selective toward KB cell lines than their enantiopure forms. Keeping in view this fact, we synthesized a few analogues (**56-63**) having 4-methyl, 4-methoxy, 4-chloro group as R at C-1 phenyl ring of tetrahydro-β-carboline ring system and alkyl amino groups such as N- methylpiperazino, propylamino and ethylamino groups as R_1, starting from methyl ester of DL tryptophan. Cytotoxicity evaluation of (**56-63**) led us to discovery of compounds **57**, **59** and **63**, which are selectively cytotoxic towards KB cells with IC_{50} values of 105.8, 664.7, 122.2 nM, respectively, and in accordance to previous SAR their cis isomers were inactive. Compound **57** having 4-methyl group as R and *N*-methylpiperazino group as R_1 combination is selectively cytotoxic towards KB cells, while compound **30** having the combination of 4-methoxy group as R and *N*-methylpiperazino group as R_1 was not selectively cytotoxic. Both compound **59** and **63** have propylamino group as R_1, but differs in R group. In this case 4-methoxy and propylamino group combination as R and R_1 was proven to be more active than 4-methyl and propylamino group combination, respectively.

Replacing β-carboline with tryptamine in **57** resulted in formation of 2,4,6-triamino-1,3,5-triazine **64**, which was found to be inactive, indicating that β-carboline part is essential for cytotoxicity. Substituting all three chloro groups of cyanuric chloride with *N*-methyl piperazine, another inactive compound **65** was obtained, proving our hypothesis correct. More selectivity of racemic mixture in comparison to pure enantiomer indicates that both the enantiomers may be acting on different targets or different sites of a target and their cumulative effect leads to selectivity, while one enantiomer alone is not selective at all. Further studies are required to delineate the mode of action and subsequent improvement in selectivity toward cancer cells.

2.3.1 Arrest of MCF-7 and MDA-MB-231 Cells in G1 and Inhibition of Mitosis:

To understand the mechanism of cell death, compound **38** was evaluated for its effect on cell cycle. Breast cancer cells MCF-7 and MDA-MB-231 were exposed to two concentrations of 0.50 and 0.74 μM for 24 hours, which were then stained with propidium iodide and analyzed by flow cytometry. DNA contents of the live population of MCF-7 cells were 54.35, 0.01, and 45.64 for untreated cells to 76.68,

0.12 and 23.20% for the cells treated with 0.74 µM of compound **38** after 24 hours in G1, S and G2 phase, respectively. (Figure 3) While, at a concentration of 0.50 µM, 64.4 % accumulation of MCF-7 cells was observed in comparison to that of 54.4 % of untreated control cells. Similar results were obtained with MDA-MB 231 cells but increase in percentage accumulation in G1 phase was less in comparison to that of MCF-7 cells. Hence, compound **38** at 0.74 µM concentration enhances mitotic arrests in G_1 phase.

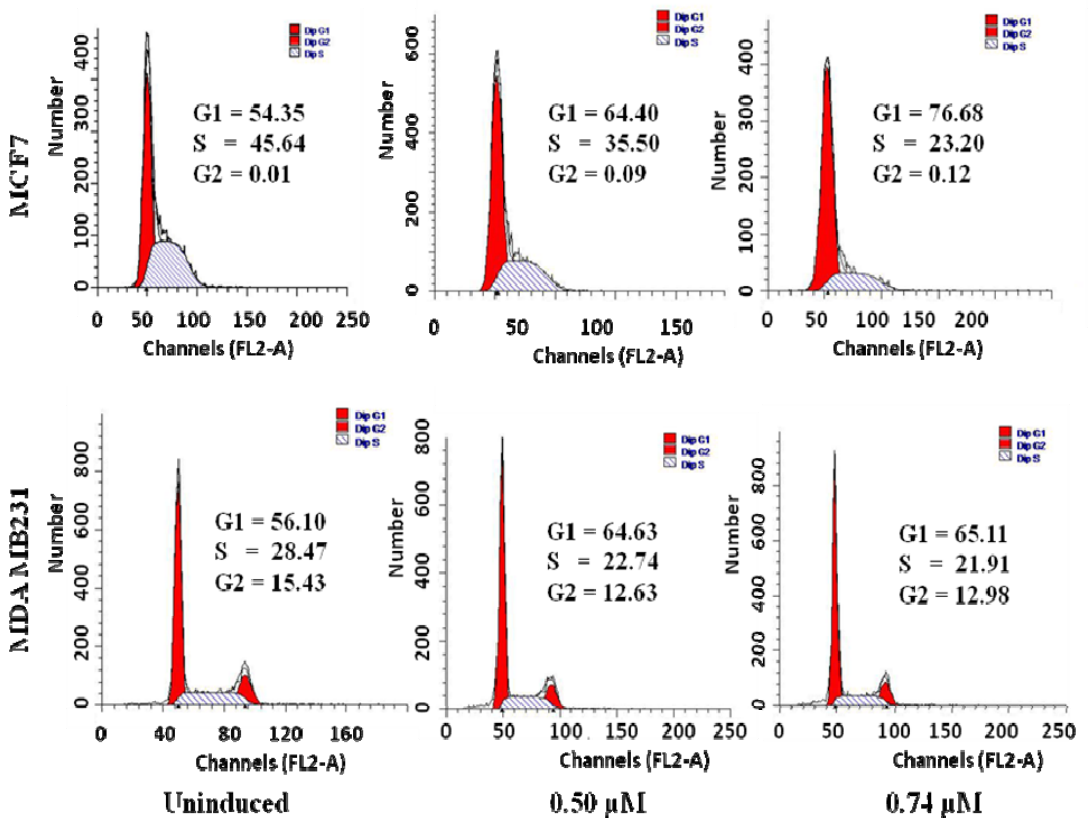


Figure 3. Effect on the cell cycle as determined by flow cytometry. Uninduced MCF-7 and MDA-MB-231 cells were taken as controls. Cells were treated with 0.50 and 0.74 µM concentrations of compound **38.**

2.3.2 Hoechst Staining for Visualization of Apoptosis:

For visualization of apoptosis both MCF-7 and MDA-MB-231 cells were treated with 0.50 and 0.74 µM concentration of compound **38** for 24 h and Hoechst staining was performed. Compound **38** causes apoptosis, was demonstrated by unevenly displayed and fragmented micronuclei in significant number of cells. (Figure 4)

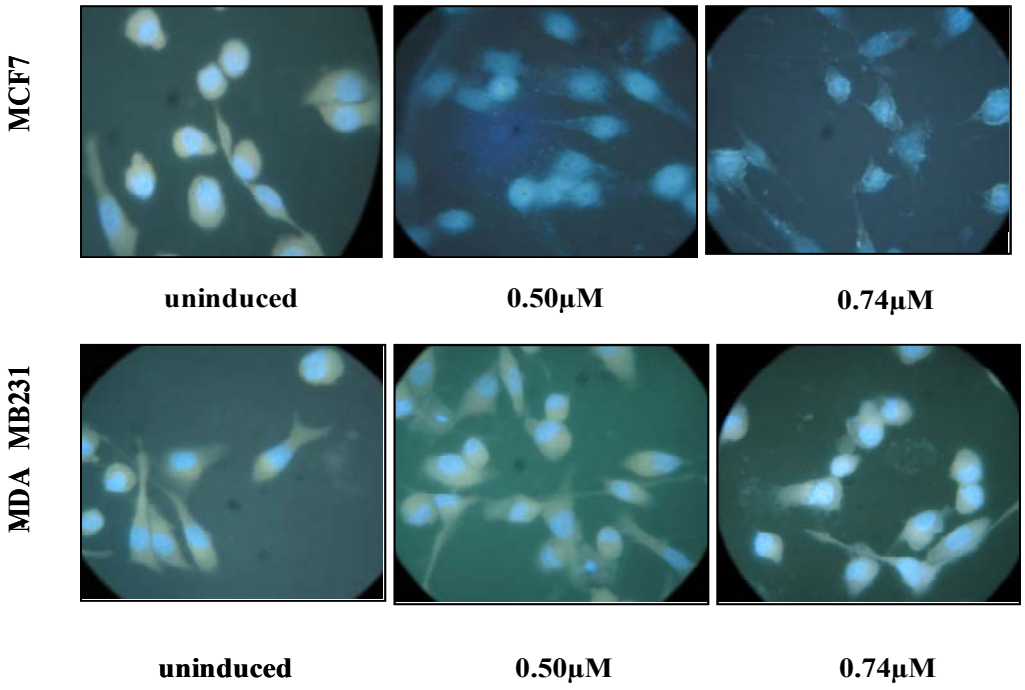


Figure 4. Hoestch staining for visualization of apoptosis. Untreated MCF-7 and MDA-MB-231 cells were taken as control.

2.3.3 DNA Fragmentation Analysis:

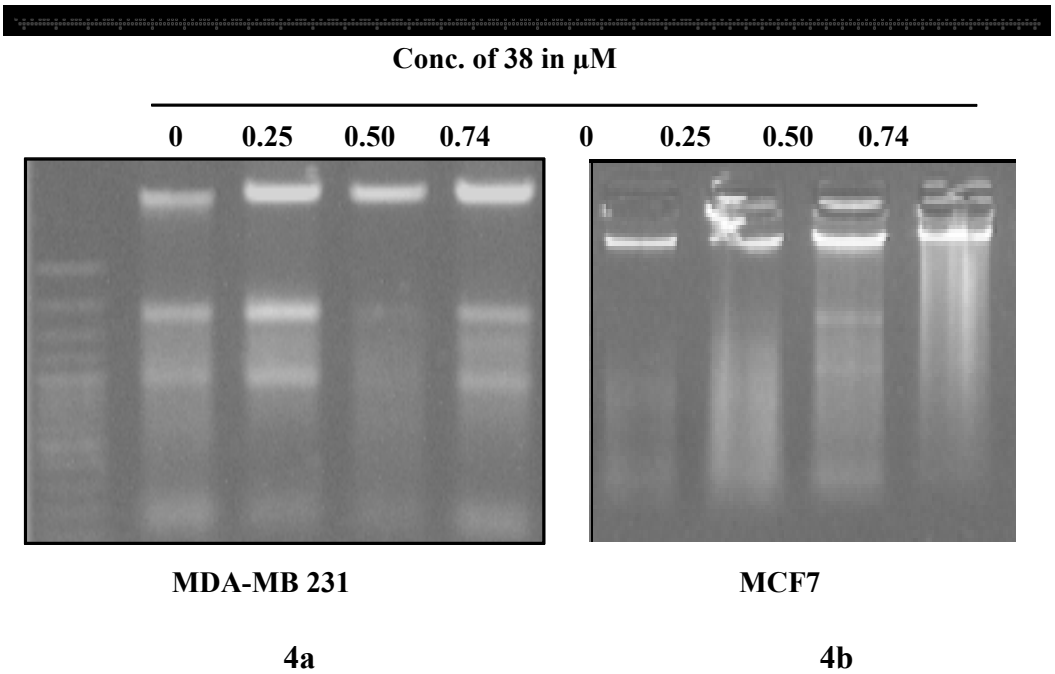


Figure 5. DNA fragmentation analysis. Depiction of DNA fragmentation pattern observed with treatment of MDA-MB-231 and MCF-7 cells with **38** at conc. of 0.5, 0.74 µM concentrations, respectively.

A nuclear associated event in apoptosis is the degradation of DNA into nucleosomal sized fragments of approximately 180-200bp which forms a ladder pattern when subjected to gel electrophoresis and is indeed one of the important diagnostic biomarker of apoptosis. Both MCF-7 and MDA-MB-231 cells were treated with varying concentrations of compound **38** ranging from 0.25 to 0.74 μM for 24 h. DNA fragmentation analysis was carried out to evaluate apoptosis in both cell lines. DNA fragmentation was observed in both cell lines at all the concentrations tested. (Figure 5)

2.4 Conclusion:

In summary, we have described a series of (tetrahydro-β-carboline)-1,3,5-triazine hybrid molecules, some of them have shown good cytotoxicity against a panel of human cancer cell lines. Compounds **57, 59** and **63** are selectively toxic towards KB (oral cancer) cell line with IC_{50} values of 105.8, 664.7 and 122.2 nM, respectively, while their enantiopure forms are less active and not selective. Compound **38,** however, is not as good as **57, 59** or **63** but is 2.5 times more cytotoxic towards MCF-7 cells than normal fibroblasts NIH3T3 with an IC_{50} value of 740 nM. Compound **38** arrests the cell cycle in G1 phase in MCF-7 as well as MDA-MB-231 cell lines, ultimately leading to cell death which was confirmed by DNA fragmentation analysis and Hoestch staining. *In vivo* studies, additional SAR, further lead optimization, and studies regarding mode of action are progress in our laboratories. These (tetrahydro-β-carboline)-1,3,5-triazine hybrids should be considered as important lead compounds for potential application in anticancer chemotherapy.

2.5 Experimental:

All non-indigenous chemical were purchased from Sigma-Aldrich. All reactions were monitored by thin layer chromatography conducted on E. Merck TLC plates (Silica gel 60 F-254, Aluminium Back) and visualized with UV light or iodine. The crude products were purified by column chromatography using silica gel as adsorbent. Nuclear magnetic spectra were recorded on 200 MHz and 300 MHz spectrometers. In the case of multiplets, the signals are reported as intervals. Signals were abbreviated as s, singlet; d, doublet; t, triplet; m, multiplet. The electron spray mass spectra were

recorded on a triple quadrupole mass spectrometer. The samples (dissolved in suitable solvents such as methanol/acetonitrile/water) were introduced into the ESI source through a syringe pump at the rate of 5 μL/min. The ESI capillary was set at 3.5 kV, and the cone voltage was 40 V. The spectra were collected in 6 s scans, and the printouts are averaged spectra of 6-8 scans. Infrared spectra were recorded using a Perkin-Elmer RX-1 spectrometer; the values were reported in cm^{-1}.

General Procedure for synthesis of compounds (12-28)

To the stirred solution of 2.04 g (10 mmol) DL/L/D-tryptophan in methanol at 0 °C, 1.34 mL (1.1 eq., 11mmol) of thionyl chloride was added drop-wise. The reaction mixture was then refluxed for 2 h. An appropriate aromatic aldehydes (1.1 eq.) were added to the reaction mixture and further refluxed for 8 h. The solvent was removed under vacuum and the solid residue so resulted was dissolved in water, neutralized with saturated sodium bicarbonate solution and extracted with ethyl acetate. The organic layer was washed with brine solution (three times), water (three times), and dried over sodium sulfate. The solvent was removed *in vacuo* and the cis and trans isomers were separated by flash column chromatography using silica gel as adsorbent. Spectroscopic data of the obtained tetrahydro-β-carbolines were identical to that of reported earlier. [24, 27]

General Procedure for synthesis of compounds (29-63)

To the stirred mixture of cyanuric chloride (0.92 g, 5 mmol) and K_2CO_3 (0.56 g, 5 mmol) in dry THF (50 mL) at 0°C was added the solution of tetrahydro-β-carboline (5 mmol) in dry THF (30 mL) drop wise for 1 h. After completion of the reaction, solvent was evaporated under vacuum and solid mass was obtained. The solid residue was dissolved in $CHCl_3$ (100 mL), washed with water (three times), dried over anhydrous Na_2SO_4, concentrated *in vacuo* and used further without purification. Then 2 mmol of resulting solid, 4 mmol of required amine and K_2CO_3 (0.55 g, 4 mmol) in dry THF (40 mL) were refluxed for 3-6 h. The reaction mixture was filtered and evaporated the solvent under vacuum. The solid residue was dissolved in $CHCl_3$ (75 mL), washed with water (three times), dried over anhydrous Na_2SO_4, purified with column chromatography using silica-gel as adsorbent.

(±)cis-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (29)

Yield: 67%; mp. 220 - 222°C; ESMS: 612 (M+1); IR (KBr): 3402, 3182, 2936, 2848, 2802, 1737, 1542, 1436, 1362 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.63 (bs, 1H), 7.59-7.13 (m, 7H), 6.57 (d, 2H, J = 8.7Hz), 6.22 (dd, 1H, J = 6.3, 2.4Hz), 3.78 (t, 8H, J = 4.9Hz), 3.75 (s, 3H), 3.61-3.54 (m, 2H), 3.06 (s, 3H), 2.43 (t, 8H, J = 5.4Hz), 2.31 (s, 6H). ^{13}C NMR ($CDCl_3$, 50MHz): 21.62, 43.44, 46.63, 50.03, 51.55, 51.97, 55.32, 55.72, 108.92, 111.08, 113.75, 118.98, 119.57, 122.12, 127.36, 130.43, 131.83, 134.03, 136.74, 159.29, 165.33, 165.95, 173.19. Anal. Calcd. for $C_{33}H_{41}N_9O_3$: C 64.79, H 6.76, N 20.61; Found: C 64.68, H 6.80, N 20.56 %.

(±)trans-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (30)

Yield: 71%; mp. 235-237°C; ESMS: 612 (M+1); IR (KBr): 3368, 3181, 2936, 2849, 2798, 1739, 1544, 1428, 1354 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.75 (bs, 1H), 7.56-6.85 (m, 7H), 6.84 (d, 2H, J = 8.1Hz), 4.41 (dd, 1H, J = 8.7, 4.1Hz), 3.78 (s, 3H), 3.76 (t, 8H, J = 4.5Hz), 3.57 (s, 3H), 3.49-3.07 (m, 2H), 2.37 (t, 8H, J = 5.6Hz), 2.29 (s, 6H). ^{13}C NMR ($CDCl_3$, 50MHz): δ (ppm) 22.44, 43.53, 46.55, 52.18, 53.63, 55.32, 55.70, 56.15, 109.95, 111.23, 114.25, 118.78, 119.82, 122.22, 127.39, 129.67, 133.74, 133.97, 136.77, 159.51, 165.29, 166.67, 172.39. Anal. Calcd. for $C_{33}H_{41}N_9O_3$: C 64.79, H 6.76, N 20.61; Found: C 64.62, H 6.74, N 20.58 %.

(±)cis-2-[4,6-Bis-(butylamino)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetra-hydro-1*H*-β-carboline-3-carboxylic acid methyl ester (31)

Yield: 65 %; mp. 132-134°C (dec.); FAB-MS: 558 (M+1); IR (KBr): 3415, 3339, 3025, 2953, 2837, 1733, 1595, 1489, 1375 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.71 (bs, 1H), 7.60-7.13 (m, 7H), 6.75 (d, 2H, J = 8.6Hz), 6.27 (dd, 1H, J = 6.2, 2.1 Hz), 3.75 (s, 3H), 3.49 (s, 3H), 3.39-3.12 (m, 6H), 1.75-1.64 (m, 4H), 1.44-1.34 (m, 4H), 0.93 (t, 6H, J = 6.1Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 14.31, 20.42, 22.85, 32.55, 40.83, 53.44, 55.47, 56.65, 57.23, 109.11, 111.34 114.21, 118.72,

119.86, 122.40, 127.29, 129.47, 134.42, 136.36, 159.82, 166.12, 166.43, 172.56. Anal. Calcd. for $C_{31}H_{39}N_7O_3$: C 66.76, H 7.05, N 17.58; Found: C 66.67, H 6.98, N 17.55 %.

(±)trans-2-[4,6-Bis-(butylamino)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (32)

Yield: 65 %; mp. 238-240°C (dec.); FAB-MS: 558 (M+1); IR (KBr); 3418, 3315, 3060, 2965, 2853, 1734, 1591, 1489, 1378 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.84 (bs, 1H), 7.56-7.15 (m, 6H), 6.87-6.79 (m, 3H), 4.57 (dd, 1H, J = 6.2, 1.9Hz), 3.76 (s, 3H), 3.59 (s, 3H), 3.52-3.44 (m, 2H), 3.39-3.21 (m, 4H), 1.50-1.27 (m, 8H), 0.94 (t, 6H, J = 6.4Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 14.29, 20.48, 22.91, 32.36, 40.85, 52.29, 54.27, 55.65, 56.30, 109.13, 111.43, 114.25, 118.70, 119.97, 122.32, 127.35, 129.30, 134.46, 136.90, 159.32, 166.29, 166.90, 172.68. Anal. Calcd. for $C_{31}H_{39}N_7O_3$: C 66.76, H 7.05, N 17.58; Found: C 66.65, H 6.94, N 17.52 %.

(±)cis-2-[4,6-Bis-(2-hydroxyethylamino)-[1,3,5]-triazin-2-yl]-1-p-tolyl-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (33)

Yield: 72 %; mp. 202-205°C (dec.); FAB-MS: 518 (M+1); IR (KBr): 3445, 3333, 3028, 2965, 2855, 1734, 1599, 1479, 1395 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.95 (bs, 1H), 7.58-7.03 (m, 9H), 6.10 (dd, 1H, J = 6.2, 2.1Hz), 3.71-3.51 (m, 8H), 3.18-3.03 (m, 2H), 3.01 (s, 3H), 2.25 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 21.44, 21.77, 43.74, 51.05, 52.00, 52.63, 62.61, 108.49, 111.37, 118.83, 119.85, 122.38, 127.06, 129.11, 129.46, 131.64, 136.73, 138.07, 165.69, 166.18, 172.92. Anal. Calcd. for $C_{27}H_{31}N_7O_4$: C 62.65, H 6.04, N 18.94; Found: C 62.57, H 6.13, N 18.85 %.

(±)trans-2-[4,6-Bis-(2-hydroxyethylamino)-[1,3,5]-triazin-2-yl]-1-p-tolyl-2,3,4,9-tetr-ahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (34)

Yield: 69 %; mp. 218-220°C; FAB-MS: 518 (M+1); IR (KBr): 3451, 3315, 3055, 2965, 2853, 1735, 1559, 1472, 1378 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.91 (bs, 1H), 7.51-7.10 (m, 8H), 6.88 (s, 1H), 4.60 (dd, 1H, J = 6.5, 2.1Hz), 3.59 (s, 3H), 3.52-3.16 (m, 10H), 2.28 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 21.25, 22.75,

43.37, 52.23, 54.33, 56.44, 62.16, 109.47, 111.30, 118.48, 119.73, 122.16, 126.84, 127.25, 129.43, 134.12, 136.65, 137.34, 138.12, 166.24, 172.63. Anal. Calcd. for $C_{27}H_{31}N_7O_4$: C 62.65, H 6.04, N 18.94; Found: C 62.62, H 6.12, N 18.86 %.

(±)trans-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(3,4,5-trimethoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (35)

Yield: 71%; mp. 169-171°C; ESMS: 672 (M+1); IR (KBr): 3415, 3058, 2937, 2849, 2797, 1741, 1658, 1542, 1432, 1353 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.78 (bs, 1H), 7.56 (d, 1H, J = 8.8Hz), 7.48 (d, 1H, J = 6.6Hz), 7.34-7.07 (m, 3H), 6.89 (s, 1H), 6.58 (d, 2H, J = 8.8Hz), 5.10 (dd, 1H, J = 6.2, 2.2Hz), 4.11 (s, 3H), 3.85-3.67 (m, 17H), 3.42-3.31 (m, 1H), 3.21-3.11 (m, 1H), 2.38-2.29 (m, 14H). ^{13}C NMR ($CDCl_3$, 50MHz): δ (ppm) 23.36, 43.35, 46.57, 51.44, 52.21, 55.28, 55.81, 56.37, 61.25, 105.74, 107.82, 111.50, 118.35, 119.72, 121.95, 122.30, 126.59, 129.33, 134.90, 136.19, 142.04, 150.33, 152.98, 165.16, 166.44, 174.72. Anal. Calcd. for $C_{33}H_{45}N_9O_5$: C 62.58, H 6.75, N 18.76; Found: C 62.47, H 6.81, N 18.73 %.

(±)cis-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(3,4,5-trimethoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (36)

Yield: 68%; mp. 142-144°C; ESMS: 672 (M+1); IR (KBr): 3372, 3062, 2936, 2847, 2799, 1741, 1593, 1435, 1357, 1279 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.38 (s, 1H), 7.64 (d, 1H, J = 8.8Hz), 7.29-7.12 (m, 4H), 6.71 (d, 2H, J = 8.8Hz), 6.23 (dd, 1H, J = 6.2, 2.1Hz), 3.95-3.77 (m, 20H), 3.62-3.53 (m, 2H), 2.42-2.25 (m, 14H). ^{13}C NMR ($CDCl_3$, 50MHz): δ (ppm) 21.67, 43.44, 46.62, 50.27, 51.97, 52.65, 55.30, 56.46, 109.16, 111.14, 119.06, 119.68, 119.96, 122.26, 127.32, 129.61, 131.42, 133.91, 136.79, 137.44, 153.28, 165.50, 165.97, 173.36. Anal. Calcd. for $C_{33}H_{45}N_9O_5$: C 62.58, H 6.75, N 18.76; Found: C 62.45; H 6.83, N 18.70 %.

(1S, 3S)-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (37)

Yield: 60%; mp. 150-152°C; ESMS: 612 (M+1); IR (KBr): 3390, 3180, 2929, 2850, 2798, 1739, 1537, 1433, 1359 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.38 (bs, 1H), 7.62-7.12 (m, 7H), 6.76 (d, 2H, J = 7.9Hz), 6.28 (dd, 1H, J = 6.1, 2.6Hz), 3.78 (s, 3H), 3.75 (t, 8H, J = 4.8Hz), 3.61-3.54 (m, 1H), 3.18-3.09 (m, 1H), 3.01 (s, 3H), 2.41 (t, 8H, J = 5.9Hz), 2.29 (s, 6H); ^{13}C NMR ($CDCl_3$, 50MHz): δ (ppm) 21.62, 43.28, 46.60, 50.05, 51.56, 51.97, 55.31, 55.72, 108.08, 111.06, 113.74, 118.96, 119.52, 122.09, 129.53, 130.42, 131.82, 134.08, 136.75, 159.27, 165.29, 165.96, 173.19. Anal. Calcd. for $C_{33}H_{41}N_9O_3$; C 64.79, H 6.76, N 20.61; Found: C 64.66, H 6.72, N 20.57%.

(1R, 3S)-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (38)

Yield: 68%; mp. 110-112°C; ESMS: 612 (M+1); IR (KBr): 3389, 3180, 2937, 2854, 2798, 1739, 1542, 1442, 1357 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.16 (bs, 1H), 7.56-7.06 (m, 8H), 6.84 (d, 1H, J = 8.2Hz), 4.48 (dd, 1H, J = 7.4, 3.3Hz), 3.78 (s, 3H), 3.75 (t, 8H, J = 4.2Hz), 3.58 (s, 3H), 3.17-3.10 (m, 2H), 2.40 (t, 8H, J = 5.1Hz), 2.31 (s, 6H). ^{13}C NMR ($CDCl_3$, 50MHz) : δ (ppm) 22.45, 43.44, 46.52, 52.18, 53.66, 55.26, 55.69, 56.17, 109.83, 111.23, 114.24, 118.75, 119.79, 122.20, 127.36, 129.63, 133.77, 133.97, 136.77, 159.49, 165.24, 166.67, 172.38. Anal. Calcd. for $C_{33}H_{41}N_9O_3$: C 64.79, H 6.76, N 20.61; Found: C 64.67, H 6.69, N 20.54%.

(1S, 3S)-2-[4,6-Bis-(4-phenylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (39)

Yield: 72%; mp. 218-220°C; FAB-MS: 736 (M+1); IR (KBr): 3340, 3056, 2944, 2829, 1739, 1597, 1544, 1438, 1370, 1174 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) 7.73 (bs, 1H), 7.36-6.76 (m, 19H), 6.24 (dd, 1H, J = 6.7, 2.3Hz), 3.99 (t, 8H, J = 4.8Hz), 3.75 (s, 3H), 3.61-3.39 (m, 2H), 3.22 (t, 8H, J = 5.1Hz), 3.04 (s, 3H); ^{13}C NMR ($CDCl_3$, 50MHz): δ (ppm) 21.82, 43.67, 49.92, 50.48, 51.93, 52.08, 55.74, 109.33, 111.21, 113.86, 117.01, 119.01, 119.97, 120.62, 122.47, 127.35, 129.65, 130.35, 131.78, 133.77, 136.68, 151.86, 159.36, 165.29, 165.63, 166.09, 173.20.

Anal. Calcd. for $C_{43}H_{45}N_9O_3$: C 70.18, H 6.16, N 17.13; Found C 70.07, H 6.19, N: 16.99%.

(1R, 3S)-2-[4,6-Bis-(4-phenylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (40)

Yield: 75%; mp. 168-170°C; FAB-MS: 736 (M+1); IR (KBr): 3338, 3146, 2945, 2848, 1730, 1593, 1519, 1433, 1352, 1168 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 7.84 (bs, 1H), 7.35-6.83 (m, 19H), 4.53 (dd, 1H, J = 7.6, 4.7Hz), 3.88 (t, 8H, J = 4.5Hz), 3.78 (s, 3H), 3.59 (s, 3H), 3.53-3.46 (m, 2H), 3.15 (t, 8H, J = 5.1Hz); ^{13}C NMR ($CDCl_3$, 50MHz): δ (ppm) 22.67, 43.59, 49.85, 52.30, 54.01, 55.72, 56.34, 109.72, 119.35, 113.74, 114.36, 116.95, 118.81, 120.12, 120.56, 122.48, 127.37, 129.27, 129.61, 134.10, 136.81, 151.85, 159.48, 165.55, 166.86, 172.62. Anal. calcd. for $C_{43}H_{45}N_9O_3$: C 70.18, H 6.16, N 17.13; Found: C 70.03, H 6.15, N: 17.19%.

(1S, 3S)-2-[4,6-Bis-(morpholin-4yl)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (41)

Yield: 73%; mp. 203-205°C; FAB-MS: 586 (M+1); IR (KBr): 3317, 2924, 2850, 1737, 1539, 1436, 1257, cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 7.69 (bs, 1H), 7.60 (d, 1H, J = 5.6 Hz), 7.32-7.13 (m, 6H), 6.77 (d, 2H, J = 8.5 Hz), 6.19 (dd, 1H, J = 6.2, 2.1Hz), 3.95-3.63 (m, 22H), 3.07-3.03 (m, 4H); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 21.77, 43.92, 44.18, 50.47, 52.05, 55.71, 67.26, 107.91, 109.19, 111.22, 113.82, 114.56, 118.96, 119.95, 122.46, 127.29, 128.68, 129.20, 130.28, 131.74, 133.67, 136.69, 159.35, 165.70, 172.33. Anal. Calcd. for $C_{31}H_{35}N_7O_5$: C 63.58, H 6.02, N 16.74; Found: C 63.46, H 6.09, N 16.71 %.

(1R, 3S)-2-[4,6-Bis-(morpholin-4yl)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (42)

Yield: 76%; mp. 203-205°C; FAB-MS: 586 (M+1); IR (KBr): 3346, 2923, 2852, 1726, 1550, 1442, 1357, 1259, 1172 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 7.85 (bs, 1H), 7.55 (d, 1H, J = 8.1Hz), 7.31-7.10 (m, 5H), 6.88 (t, 3H, J = 6.7Hz), 4.66 (dd, 1H, J = 6.8, 2.1Hz), 3.80 (s, 3H), 3.69 (bs, 8H), 3.60 (s, 3H), 3.55 (bs, 8H), 3.52-3.46 (m, 1H), 3.22-3.15 (m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 22.61, 44.11, 52.25, 53.93, 55.69,

56.34, 67.25, 109.66, 111.33, 114.31, 118.77, 120.09, 122.48, 127.32, 129.24, 134.01, 136.85, 137.55, 159.46, 165.30, 166.72, 172.54; Anal. Calcd. for $C_{31}H_{35}N_7O_5$: C 63.58, H 6.02, N 16.74; Found: C 63.44, H 5.98, N 16.83 %.

(1R, 3S)-2-[4,6-Bis-(cyclohexylamino)-1,3,5-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (43)

Yield: 65%; mp. 151-153°C; FAB-MS: 610 (M+1); IR (KBr): 3203, 3082, 2931, 2858, 1735, 1558, 1454, 1352, 1245, 1170 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 7.83 (bs, 1H), 7.57-6.75 (m, 9H), 4.74 (dd, 1H, J = 7.5, 2.2Hz), 3.79 (s, 3H), 3.65 (s, 3H), 3.12-2.92 (m, 2H), 1.94-1.18 (m, 22H); ^{13}C NMR ($CDCl_3$, 50MHz): δ (ppm) 24.78, 30.11, 32.37, 33.63, 49.41, 50.23, 52.21, 54.44, 55.67, 111.41, 113.40, 114.10, 114.27, 118.72, 119.70, 121.70, 127.30, 127.53, 131.24, 136.71, 137.16, 158.26, 159.05, 166.45, 179.74. Anal. Calcd. for $C_{35}H_{43}N_7O_3$: C 68.94, H 7.11, N 16.08; Found: C 68.87, H 6.92, N 16.14 %.

(1S, 3S)-2-[4,6-Bis-(o-tolylamino)-1,3,5-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (44)

Yield: 64%; mp. 188-190°C; ESMS: 626 (M+1); IR (KBr): 3267, 2925, 2839, 1739, 1591, 1452, 1245 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 7.85 (bs, 1H), 7.57-7.17 (m, 17H), 6.66 (dd, 1H, J = 6.4, 2.5Hz), 3.74 (s, 3H), 3.72 (s, 3H), 3.56-3.34 (m, 2H), 2.17 (s, 6H); ^{13}C NMR ($CDCl_3$, 50MHz): δ (ppm) 18.57, 21.66, 50.41, 52.15, 52.41, 55.67, 109.15, 111.21, 113.63, 118.99, 119.99, 122.55, 123.98, 125.03, 126.73, 127.18, 130.11, 131.48, 132.76, 136. 72, 137.17, 159.40, 165.22, 166.45, 172.75. Anal. Calcd. for $C_{37}H_{35}N_7O_3$: C 71.02, H 5.64, N 15.67; Found: C 70.93, H 5.67, N 15.69 %.

(1R, 3S)-2-[4,6-Bis-(o-tolylamino)-1,3,5-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (45)

Yield: 70%; mp. 188-190°C; FAB-MS: 640 (M+1); IR (KBr): 3244, 2949, 2842, 1741, 1569, 1515, 1448, 1244 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 7.91 (bs, 1H), 7.53-7.06 (m, 17H), 4.88 (dd, 1H, J = 6.4, 2.5Hz), 3.75 (s, 3H), 3.72 (s, 3H), 3.56-3.35 (m, 2H), 2.18 (s, 6H); ^{13}C NMR ($CDCl_3$, 50MHz): δ (ppm) 19.82, 21.49, 40.20, 42.71, 50.54, 56.51, 57.18, 107.83, 110.00, 111.46, 111.62, 114.25, 118.75,

120.22, 122.56, 124.98, 126.18, 126.90, 127.04, 128.73, 129.73, 131.07, 136. 27, 142.78, 159.39, 165.63, 169.42, 173.56. Anal. Calcd. for $C_{37}H_{35}N_7O_3$: C 71.02, H 5.64, N 15.67; Found: C 70.89, H 5.56, N 15.70 %.

(1R, 3S)-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(3,4,5-trimethoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (46)

Yield: 67%; mp. 148-150°C; ESMS: 672 (M+1); IR (KBr): 3364, 3060, 2937, 2851, 2799, 1739, 1658, 1549, 1439, 1352 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.72 (s, 1H), 7.56-7.06 (m, 6H), 6.92 (s, 1H), 4.65 (dd, 1H, J = 6.1, 2.2 Hz), 3.72 (bs, 17H), 3.57 (s, 3H), 3.51-3.44 (m, 1H), 3.23-3.13 (m, 1H), 2.34 (t, 8H, J = 5.9Hz), 2.21 (s, 6H). ^{13}C NMR ($CDCl_3$, 50MHz): 21.64, 43.41, 46.60, 50.25, 51.97, 52.68, 55.32, 56.45, 109.15, 111.13, 119.05, 119.67, 119.97, 122.27, 127.30, 129.67, 133.82, 131.41, 136.79, 137.45, 153.29, 165.49, 165.96, 173.38. Anal. Calcd. for $C_{33}H_{45}N_9O_5$: C 62.58, H 6.75, N 18.75; Found: C 62.52, H 6.78, N 18.64 %.

(1S, 3S)-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(3,4,5-trimethoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (47)

Yield: 65%; mp. 140-142°C; ESMS: 672 (M+1); IR (KBr): 3373, 3061, 2936, 2848, 2798, 1739, 1593, 1435, 1357, 1278 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.36 (s, 1H), 7.62 (d, 1H, J = 8.7Hz), 7.29-7.13 (m, 4H), 6.70 (d, 2H, J = 8.8Hz), 6.22 (dd, 1H, J = 6.2, 2.3Hz), 3.94-3.78 (m, 20H), 3.61-3.51 (m, 2H), 2.42-2.25 (m, 14H). ^{13}C NMR ($CDCl_3$, 50MHz): δ (ppm) 21.64, 43.41, 46.60, 50.25, 51.97, 52.67, 55.32, 56.45, 109.15, 111.12, 119.05, 119.66, 119.97, 122.27, 127.30, 129.62, 131.41, 133.91, 136.79, 137.45, 153.29, 165.49, 165.96, 173.38. Anal. Calcd. for $C_{33}H_{45}N_9O_5$: C 62.58, H 6.75, N 18.75; Found: C 62.51; H 6.82, N 18.73 %.

(1R, 3S)-2-[4,6-Bis-(4-ethylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (48)

Yield: 72%; mp. 162-164°C; ESMS: 640 (M+1); IR (KBr): 3401, 3183, 2935, 2813, 1739, 1550, 1439, 1350 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.57 (bs, 1H),

7.56 (d, 1H, J = 8.8 Hz), 7.35-7.27 (m, 3H), 7.20-7.09 (m, 2H), 7.01 (s, 1H), 6.86 (d, 2H, J = 8.8 Hz), 4.48 (dd, 1H, J = 8.9, 3.0Hz), 3.80 (s, 3H), 3.76 (t, 8H, J = 4.9Hz), 3.60 (s, 3H), 3.56-3.46 (m, 1H), 3.18-3.12 (m, 1H), 2.43 (bs, 12H), 1.11 (t, 6H, J = 6.0 Hz). ^{13}C NMR ($CDCl_3$, 50MHz): δ (ppm) 12.13, 22.48, 43.40, 52.15, 52.77, 53.11, 53.71, 55.69, 56.16, 109.91, 111.18, 114.25, 118.76, 119.85, 122.25, 127.39, 129.56, 133.91, 136.73, 159.51, 165.13, 166.68, 172.40. Anal. Calcd. for $C_{35}H_{45}N_9O_3$: C 65.71, H 7.09, N 19.70; Found: C 65.62, H 7.14, N 19.65 %.

(1R, 3S)-2-[4,6-Bis-(4-propylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (49)

Yield: 72%; mp. 158-160°C; ESMS: 668 (M+1); IR (KBr): 3400, 3184, 2935, 2811, 1740, 1547, 1439, 1352 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.50 (bs, 1H), 7.57 (d, 1H, J = 6.4 Hz), 7.35-7.27 (m, 3H), 7.20-7.12 (m, 2H), 7.01 (s, 1H), 6.86 (d, 2H, J = 8.8Hz), 4.48 (dd, 1H, J = 6.4, 2.1 Hz), 3.80 (s, 3H), 3.76 (t, 8H, J = 4.9Hz), 3.60 (s, 3H), 3.54-3.46 (m, 1H), 3.18-3.12 (m, 1H), 2.43 (t, 8H, J = 5.7 Hz), 2.29 (t, 4H, J = 5.9Hz), 1.54 (m, 4H), 1.11 (t, 6H, J = 6.0Hz). ^{13}C NMR ($CDCl_3$, 50MHz): 12.32, 20.17, 22.48, 43.37, 52.15, 53.53, 55.68, 56.16, 61.15, 71.83, 109.94, 111.18, 114.27, 118.77, 119.84, 122.24, 127.40, 129.58, 133.91, 136.73, 159.49, 165.14, 166.67, 172.40. Anal. Calcd. for $C_{37}H_{49}N_9O_3$: C 66.54, H 7.40, N 18.88; Found: C 66.47, H 7.44, N 18.91 %.

(1R, 3R)-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (50)

Yield: 63%; mp. 125-127°C; ESMS: 612 (M+1); IR (KBr): 3392, 3180, 2928, 2850, 2796, 1739, 1537, 1432, 1359 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.70 (bs, 1H), 7.62-7.12 (m, 7H), 6.76 (d, 2H, J = 8.5Hz), 6.20 (dd, 1H, J = 6.1, 2.6Hz), 3.80 (s, 3H), 3.75 (t, 8H, J = 4.8Hz), 3.53-3.10 (m, 2H), 3.07 (s, 3H), 2.61 (t, 8H, J = 5.4Hz), 2.30 (s, 6H). ^{13}C NMR ($CDCl_3$, 50MHz): δ (ppm) 21.64, 43.38, 46.59, 50.13, 51.63, 51.96, 55.31, 55.71, 108.95, 111.07, 113.75, 118.96, 119.60, 122.14, 129.43, 130.40, 131.83, 134.03, 136.73, 159.29, 165.55, 165.55, 173.19. Anal. Calcd. for $C_{33}H_{41}N_9O_3$: C 64.79, H 6.76, N 20.61; Found: C 64.67, H 6.84, N 20.59 %.

(1S, 3R)-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (51)

Yield: 71%; mp. 125-128°C; ESMS: 612 (M+1); IR (KBr): 3372, 3180, 2937, 2849, 2797, 1739, 1548, 1441, 1356 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.40 (bs, 1H), 7.56-7.09 (m, 6H), 6.97 (s, 1H), 6.84 (d, 2H, J = 8.4Hz), 4.47 (dd, 1H, J = 7.7, 4.1Hz), 3.78 (s, 3H), 3.75 (t, 8H, J = 4.2Hz), 3.54 (s, 3H), 3.49-3.46 (m, 1H), 2.64-2.58 (m, 1H), 2.35 (t, 8H, J = 5.8Hz), 2.27 (s, 6H). ^{13}C NMR ($CDCl_3$, 50MHz): δ (ppm) 22.50, 43.43, 46.53, 53.77, 54.63, 55.31, 55.70, 56.18, 109.82, 111.23, 114.25, 118.76, 119.89, 122.27, 127.37, 129.47, 133.94, 136.77, 159.46, 161.14, 165.26, 166.65, 172.43. Anal. Calcd. for $C_{33}H_{41}N_9O_3$: C 64.79, H 6.76, N 20.61; Found: C 64.73, H 6.81, N: 20.56 %.

(1R, 3R)-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-phenyl-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (52)

Yield: 73%; mp. 120-122°C; ESMS: 582 (M+1); IR (KBr): 3405, 3062, 2937, 2850, 2798, 1738, 1539, 1489, 1436, 1363 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.43 (s, 1H), 7.62 (d, 1H, J = 7.8Hz), 7.45-7.14 (m, 9H), 6.22 (dd, 1H, J = 7.8, 2.5Hz), 3.84 (t, 8H, J = 6.1Hz), 3.63-3.56 (m, 1H), 3.22-3.02 (m, 1H), 2.98 (s, 3H), 2.43-2.26 (m, 14H). ^{13}C NMR ($CDCl_3$, 50MHz): δ (ppm) 21.64, 43.26, 46.63, 50.18, 51.82, 52.14, 55.29, 108.99, 111.05, 119.01, 119.58, 122.15, 127.33, 127.88, 128.54, 129.17, 131.47, 136.75, 141.83, 165.30, 166.04, 173.09. Anal. Calcd. for $C_{32}H_{39}N_9O_2$: C 66.07, H 6.76, N 21.67; Found: C 66.02, H: 6.88, N 21.62 %.

(1S, 3R)-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-phenyl-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (53)

Yield: 75%; mp. 144-146°C; ESMS: 582 (M+1); IR (KBr): 3394, 2936, 2851, 2799, 1739, 1548, 1437, 1356 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.18 (s, 1H), 7.56 (d, 1H, J = 8.1Hz), 7.42-7.12 (m, 8H), 6.91 (s, 1H), 4.76 (dd, 1H, J = 6.3, 2.6Hz), 3.73 (t, 8H, J = 5.9Hz), 3.60 (s, 3H), 3.56-3.48 (m, 1H), 3.24-3.18 (m, 1H), 2.44-2.29 (m, 14H). ^{13}C NMR ($CDCl_3$, 50MHz): δ (ppm) 22.60, 43.46, 46.45, 52.24, 54.00, 55.33, 56.84, 109.53, 111.24, 118.80, 119.85, 122.26, 127.34, 127.95, 128.17, 128.54,

128.93, 133.80, 136.82, 141.75, 142.14, 165.17, 166.79, 172.51. Anal. Calcd. for $C_{32}H_{39}N_9O_2$: C 66.07, H 6.76, N 21.67; Found: C 65.96, H 6.83, N 21.61 %.

(1R, 3R)-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-p-tolyl-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (54)

Yield: 67%; mp. 150-152°C; ESMS: 596 (M+1); IR (KBr): 3425, 2937, 2849, 2800, 1738, 1589, 1541, 1436, 1355, 1277 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 9.05 (bs, 1H), 7.60-6.94 (m, 9H), 6.21 (dd, 1H, *J* = 6.8, 1.9Hz), 3.78 (t, 8H, J = 4.6Hz), 3.61-3.53 (m, 1H), 3.10-3.03 (m, 1H), 2.82 (s, 3H), 2.81-2.01 (m, 14H); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 21.46, 21.67, 43.34, 46.61, 50.25, 51.77, 51.94, 55.31, 108.85, 111.06, 118.95, 119.55, 122.08, 127.36, 128.05, 129.10, 129.58, 131.82, 133.82, 136.75, 137.44, 138.80, 165.34, 165.03, 173.16; Anal. Calcd. for $C_{33}H_{41}N_9O_2$: C 66.53, H 6.94, N 21.16; Found: C 66.42, H 6.95, N 20.98 %.

(1S, 3R)-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-p-tolyl-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (55)

Yield: 72%; mp. 138-140°C; ESMS: 596 (M+1); IR (KBr): 3368, 2937, 2852, 2798, 1739, 1549, 1439, 1353 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.68 (bs, 1H), 7.54 (d, 1H, J = 6.6 Hz), 7.30-7.09 (m, 7H), 6.95 (s, 1H), 4.52 (dd, 1H, J = 6.3, 2.2Hz), 3.70 (t, 8H, J = 4.9Hz), 3.70 (s, 3H), 3.57-3.43 (m, 1H), 3.18-3.11 (m, 1H), 2.34 (m, 17H). ^{13}C NMR ($CDCl_3$, 50MHz): δ (ppm) 21.55, 22.44, 43.55, 46.57, 52.19, 53.84, 55.39, 56.53, 109.70, 111.20, 118.77, 119.78, 122.18, 127.39, 128.29, 129.59, 133.95, 136.80, 137.69, 138.81, 165.21, 166.75, 172.44. Anal. Calcd. for $C_{33}H_{41}N_9O_2$: C 66.53, H 6.94, N 21.16; Found: C 66.38, H 6.98, N 21.18 %.

(±)trans-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(4-chlorophenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (56)

Yield: 68%; mp. 224-227ºC (dec.); ESMS: 616 (M+1); IR (KBr): 3367, 2937, 2852, 2799, 1738, 1549, 1488, 1440, 1354, 1277 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 8.35 (bs, 1H), 7.54 (d, 1H, J = 6.0Hz), 7.37-7.10 (m, 7H), 6.84 (s, 1H), 4.86 (dd, J = 7.0, 2.3Hz), 3.70 (t, 8H, J = 4.7Hz), 3.58 (s, 3H), 3.51-3.43 (m, 1H), 3.22-3.12 (m, 1H), 2.35-2.27 (m, 14H); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 22.67, 43.40, 46.54, 52.29,

54.11, 55.28, 56.29, 109.48, 111.34, 118.85, 120.02, 122.48, 127.26, 129.07, 129.35, 133.34, 133.61, 136.90, 141.06, 165.17, 166.76, 172.46. Anal. Calcd. for $C_{32}H_{38}ClN_9O_2$: C 62.38, H 6.22, N 20.46; Found: C 62.29, H 6.19, N 20.42 %.

(±)trans-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-p-tolyl-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (57)

Yield: 63%; mp. 160-162°C; FAB-MS: 596 (M+1); IR (KBr): 3387, 3078, 2936, 2855, 1739, 1543, 1443, 1359, 1259 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 8.45 (bs, 1H), 7.54-7.08 (m, 8H), 6.92 (s, 1H), 4.61 (dd, 1H, J = 7.7, 2.6Hz), 3.90 (t, 8H, J = 5.1Hz), 3.59 (s, 3H), 3.19-3.10 (m, 2H), 2.36 (t, 8H, J = 4.9Hz), 2.32 (s, 3H), 2.25 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.49, 22.63, 43.34, 46.55, 53.99, 55.31, 56.57, 57.78, 109.53, 111.24, 118.76, 119.91, 122.28, 127.39, 128.00, 129.58, 134.09, 136.82, 137.54, 139.11, 165.23, 166.81, 172.54. Anal. Calcd. for $C_{33}H_{41}N_9O_2$: C 66.53, H 6.94, N 21.16; Found: C 66.47, H 6.98, N 20.97 %.

(±)cis-2-[4,6-Bis-(propylamino)-[1,3,5]-triazin-2-yl]-1-p-tolyl-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (58)

Yield: 70 %; mp. 138-140°C; FAB-MS: 514 (M+1); IR (KBr): 3408, 3311, 3060, 2968, 2855, 1736, 1570, 1449, 1378 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.72 (bs, 1H), 7.62-7.03 (m, 9H), 6.28 (dd, 1H, J = 6.2, 1.9Hz), 3.39-3.30 (m, 4H), 3.15-3.05 (m, 2H), 2.97 (s, 3H), 2.28 (s, 3H), 1.69-1.58 (m, 4H), 0.96 (t, 6H, J = 7.3Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 11.88, 21.50, 22.95, 23.45, 42.88, 50.16, 51.83, 52.68, 109.06, 111.33, 118.90, 119.85, 122.35, 127.03, 129.08, 129.46, 131.65, 136.73, 137.52, 138.22, 165.78, 173.16; Anal. calcd. for $C_{29}H_{35}N_7O_2$: C 67.81, H 6.87, N 19.09; Found: C 67.73, H 6.96, N 19.12 %.

(±)trans-2-[4,6-Bis-(propylamino)-[1,3,5]triazin-2-yl]-1-p-tolyl-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (59)

Yield: 63 %; mp. 180-183°C; FAB-MS: 514 (M+1); IR (KBr): 3430, 3315, 3033, 2951, 2860, 1733, 1575, 1480, 1378 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.89 (bs, 1H), 7.48-7.02 (m, 8H), 6.87 (s, 1H), 4.60 (dd, 1H, J = 6.5, 2.1Hz), 3.56 (s, 3H), 3.41-3.32 (m, 4H), 3.29-3.15 (m, 2H), 2.31 (s, 3H), 1.67-1.55 (m, 4H), 0.97 (t, 6H, J = 7.2Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 11.82, 21.49, 22.95, 23.47, 42.87,

52.27, 54.39, 56.60, 109.68, 111.33, 118.73, 119.99, 122.31, 126.74, 127.82, 129.57, 134.41, 136.85, 137.44, 138.55, 162.85, 166.35, 172.73; Anal. Calcd. for $C_{29}H_{35}N_7O_2$: C 67.81, H 6.87, N 19.09; Found: C 67.78, H 6.75, N 19.23 %.

(±)trans-2-[4,6-Bis-(ethylamino)-[1,3,5]-triazin-2-yl]-1-p-tolyl-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (60)

Yield: 65%; mp. 232-234°C (dec.); FAB-MS: 486 (M+1); IR (KBr): 3439, 3315, 3035, 2975, 2865, 1735, 1580, 1483, 1385 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.86 (bs, 1H), 7.46-7.02 (m, 8H), 6.92 (s, 1H), 4.56 (dd, 1H, J = 6.6, 2.1Hz), 3.58 (s, 3H), 3.44-3.31 (m, 4H), 3.25-3.12 (m, 2H), 2.29 (s, 3H), 1.12 (t, 6H, J = 7.2Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 15.58, 21.69, 22.60, 36.07, 52.31, 54.49, 56.78, 109.78, 111.63, 118.89, 119.73, 122.39, 126.84, 127.62, 129.88, 134.61, 136.87, 137.64, 138.45, 162.90, 166.75, 172.68; Anal. Calcd. for $C_{27}H_{31}N_7O_2$: C 66.78, H 6.43, N 20.19; Found: C 66.65, H 6.66, N 20.05 %.

(±)trans-2-[4,6-Bis-(ethylamino)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (61)

Yield: 61%; mp. 227-229°C (dec.); FAB-MS: 502 (M+1); IR (KBr); 3429, 3318, 3029, 2971, 2859, 1732, 1585, 1483, 1380 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.88 (bs, 1H), 7.56-7.15 (m, 6H), 6.86-6.78 (m, 3H), 4.54 (dd, 1H, J = 6.2, 2.3Hz), 3.77 (s, 3H), 3.62 (s, 3H), 3.52-3.23 (m, 6H), 1.17 (t, 6H, J = 6.8Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 15.64, 21.97, 36.11, 52.30, 54.47, 55.43, 56.79, 109.26, 111.25, 118.92, 119.87, 122.39, 127.32, 129.12, 129.37, 131.69, 136.72, 137.57, 138.37, 163.14, 165.98, 173.42; Anal. Calcd. for $C_{27}H_{31}N_7O_3$: C 64.65, H 6.23, N 19.55; Found: C 64.57, H 6.32, N 19.45 %.

(±)cis-2-[4,6-Bis-(propylamino)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetra-hydro-1*H*-β-carboline-3-carboxylic acid methyl ester (62)

Yield: 72 %; mp. 140-143°C (dec.); FAB-MS: 530 (M+1); IR (KBr): 3429, 3315, 2953, 2829, 1732, 1599, 1479, 1380 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.89 (bs, 1H), 7.59-7.15 (m, 7H), 6.83 (d, 2H, J = 8.6Hz), 6.25 (dd, 1H, J = 6.1, 2.1Hz), 3.73 (s, 3H), 3.52 (s, 3H), 3.42-3.35 (m, 4H), 3.30-3.10 (m, 2H), 1.57-1.49 (m, 4H), 0.98 (t, 6H, J = 6.8Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 15.57, 21.49, 21.62,

36.08, 50.16, 51.19, 52.01, 52.06, 109.25, 111.24, 118.95, 119.86, 122.38, 127.34, 129.09, 129.36, 131.64, 136.75, 137.58, 138.36, 165.97, 166.74, 173.11; Anal. Calcd. for $C_{29}H_{35}N_7O_3$: C 65.76, H 6.66, N 18.51; Found: C 65.72, H 6.78, N 18.44 %.

(±)trans-2-[4,6-Bis-(propylamino)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (63)

Yield: 68 %; mp. 220-223°C; FAB-MS: 530 (M+1); IR (KBr): 3419, 3340, 3060, 2959, 2870, 1734, 1562, 1459, 1353 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.89 (bs, 1H), 7.57-7.13 (m, 7H), 6.86-6.75 (m, 3H), 4.69 (dd, 1H, J = 6.3, 2.1Hz), 3.76 (s, 3H), 3.59 (s, 3H), 3.53-3.45 (m, 2H), 3.40-3.22 (m, 4H), 1.52-143 (m, 4H), 0.91 (t, 6H, J = 7.2Hz); ^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d_6): δ (ppm) 11.64, 22.76, 23.22, 42.52, 52.04, 54.20, 54.46, 56.07, 111.48, 113.92, 118.18, 119.28, 121.66, 126.92, 128.90, 134.69, 136.76, 136.92, 158.97, 165.85, 166.51, 173.16. Anal. Calcd. for $C_{29}H_{35}N_7O_3$: C 65.76, H 6.66, N 18.51; Found: C 65.63, H 6.70, N 18.40 %.

Typical procedure for synthesis of compound (64)

To 1.5 eq. of cyanuric chloride in dry THF was added dropwise a solution of tryptamine (1 eq.) at 0°C in dry THF over a period of half an hour. The reaction mixture was stirred for 1hour and solvent was removed in vacuo, washed with water and extracted with chloroform. Organic layers were combined, dried with Na_2SO_4 and solvent was evaporated under reduced pressure. Resulting solid was refluxed with 2 eq. of N-methylpiperazine in THF. When TLC analysis showed completion of reaction, solvent was evaporated and resulted solid was column chromatographed to get pure compounds.

***N*-[2-(1H-indol-3-yl)ethyl]-4,6-bis-(morpholin-4-yl)-1,3,5-triazin-2-amine (64)**

Yield: 76%; mp. 160-162°C; ESMS: 436 (M+1); IR (KBr): 2934, 2852, 2800, 1534, 1497, 1444, 1278 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 8.01 (bs, 1H), 7.62 (d, 1H, J = 7.8Hz), 7.33 (d, 1H, J = 7.8Hz), 7.19 -7.08 (m, 3H), 5.08 (s, 1H), 3.54 (t, 8H, J = 4.5Hz), 2.44 (t, 8H, J = 4.5Hz), 2.31 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 26.18, 41.43, 43.36, 46.66, 55.39, 111.59, 113.85, 119.26, 119.61, 122.41, 123.02,

127.89, 136.82, 165.63, 166.76; Anal. Calcd. for $C_{23}H_{33}N_9$: C 63.42, H 7.64, N 28.94; Found: C 63.25, H 7.66, N 28.97 %.

Typical procedure for synthesis of compound (65)

To a solution of cyanuric chloride in THF was added 3.3 eq. of N-methylpiperazine and refluxed for 4 hours. Solvent was removed in vacuo and resulting solid was crystalized from ethanol to get pure compound **65**.

2,4,6-Tris(4-methylpiperazin-1-yl)-1,3,5-triazine (65)

Yield: 86%; mp. 160-162°C; ESMS: 376 (M+1); IR (KBr): 2934, 2852, 2800, 1534, 1497, 1444, 1278 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 3.21 (t, 8H, J = 4.5Hz), 2.46 (t, 8H, J = 4.5Hz), 2.28 (s, 6H); ^{13}C NMR (50 MHz,DMSO-d_6): δ (ppm) 46.66, 53.56, 58.24, 179.62; Anal. Calcd. for $C_{18}H_{33}N_9$: C 57.57, H 8.86, N 33.57; Found: C 57.42, H 8.89, N 33.45 %.

2.6 Biological assays:

MTT, PI, DAPI, guanosine 5'-triphosphate (GTP), and PIPES were obtained from Sigma, U.S.A. DMEM (Dulbecco's modified eagles medium) and fetal bovine serum (FBS) were procured from Gibco BRL, U.S.A. DMSO was from Merck, India, and antibiotic solution (containing penicillin and streptomycin) was obtained from Hyclone, U.S.A. Human cancer cell lines representing breast cancer (MCF-7), colon (SW620), prostate (DU-145), oral (KB), ovary (PA1), Leukemia (K562), pancreas (MiaPaCa-2), Lung (A-549) and normal fibroblasts (NIH3T3) were procured from NCCS, Pune, India.

2.6.1 Cytotoxicity assay:

Various concentrations of (tetrahydro-β-carboline)-1,3,5-triazine hybrids were tested for *in vitro* cytotoxic activity on human cancer cell lines representing breast cancer (MCF-7), colon (SW620), prostate (DU-145), oral (KB), ovary (PA1), leukemia (K562), pancreas (MiaPaCa-2), lung (A-549) and normal fibroblasts (NIH3T3). Cytotoxicity was measured by MTT assay, which is based on the principle of uptake of MTT by the metabolically active cells, where it is metabolized by active mitochondria into a blue-colored formazan product that is read

spectrophotometrically.[27] Briefly, tumor cells were seeded (5000-10000 cells/well) in 96-well culture plates and incubated at 37 °C in a CO_2 incubator with various concentrations of (tetrahydro-β-carboline)-1,3,5-triazine hybrids analogues ranging from 1 to 100 μg/mL, with relevant controls in triplicate wells. After 72 h, the assay was terminated by the addition of 25 μL of MTT solution (5 mg/mL) in each well. Percentage cytotoxicity was calculated as given below. The IC_{50} values were determined by nonlinear regression using Prism software v 4.01.

percentage cytotoxicity = $100 \times [1 - (X/R_1)]$

where X = absorbance of treated sample at 540 nm and R_1 = absorbance of control sample at 540 nm.

2.6.2 Analysis of Cell Cycle by Flow Cytometry:

For cell cycle analysis of MCF-7 and MDA-MB-231 cells, 1×10^5 cells/mL were plated in 6 well plate. After treatment of cells with the compound **38** and incubation, cells were trysinized and washed with 1X PBS, 2 mL of cold 70% ethanol was added, and cell suspension was fixed on ice for at least 30 min. Cells were centrifuged for 5 min at 1200 rpm and washed with 1X PBS. Cell pellets were resuspended in 500μl of freshly made propidium iodide (PI) staining solution (0.1% Triton X-100, 0.1% sodium citrate, 50 μg/mL PI and 10 μg/mL of RNase A in PBS (with Ca^{2+} and Mg^{2+}).[29] Cells were analysed in Flow Cytometer (FACS Calibur, Beckon Dickinson, USA).

2.6.3 Visualization of Apoptotic Bodies by Hoechst Staining:

For Hoechst staining in MCF-7 and MDA-MB-231, 1 X 10^4 cells were plated in 6 well plate on coverslip. After treatment of cells with compound **38**, they were washed with 1X PBS and fixed with 4% paraformaldehyde for 15min. After fixing cells were stained with 1.0 ug/mL of Hoechst stain for 15 min. at 4 °C. Excess stain was washed with 1X PBS, coverslips were mounted with fluorescent mounting solution. Cells were visualized under fluorescent microscope.[30]

2.6.4 Analysis of Apoptosis by DNA fragmentation:

The DNA fragmentation assay was performed according to the protocol[31] described previously by Nianyu et.al. MCF-7 and MDA-MB-231 cells were treated with

different concentration of compound **38**. After 24 hrs, 1X 10^5 cells were trypsinized and washed with PBS (4^0C, pH 7.4) and collected by centrifugation at 1200 rpm for 5 min. The pellet was then treated with 0.5 mL of lysis buffer (10 mM Tris-HCL, pH 7.4, 10 mM EDTA, 0.5% sodium dodecyl sulfate) for 10 min on ice. After treatment with RNase A (final concentration, 100 µg/mL) for 1 h at 37 °C, the cells were incubated at 50 °C for 4 h in the presence of 100 µg/mL proteinase K. DNA was precipitated by addition of 50 µl of 3 M sodium acetate (pH 5.2) and 1 mL of cold (4 °C) 100% ethanol to the solution. DNA was then collected and dissolved in TE buffer (10 mM Tris pH 8.0, EDTA 1 mM). For analysis, 10–20 µl of DNA was loaded on a 1.5% agarose gel containing 10 µg/mL ethidium bromide. DNA was visualized under ultraviolet light and photographed.

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Chapter 3

Synthesis of 2-(pyrimidin-2-yl)-1-phenyl-2,3,4,9-tetrahydro-1H-β-carbolines as antileishmanial agents

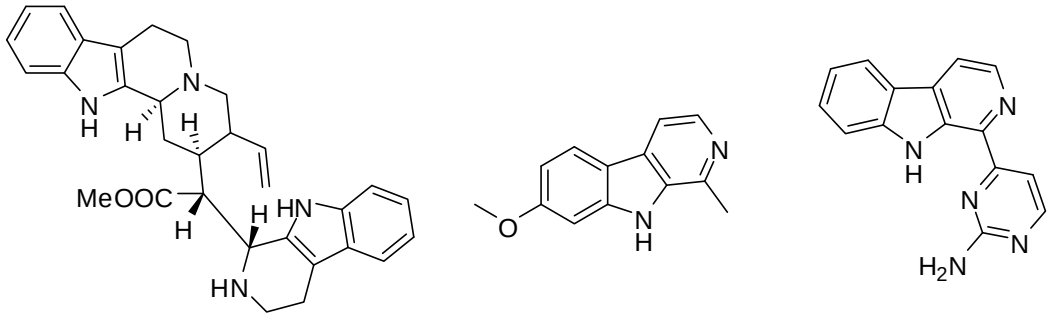
3.1 Introduction:

Leishmaniases,[1] a group of vector-borne parasitic diseases caused by several protozoan parasite of genus *Leishmania*, are endemic in large areas of the tropics, subtropics and Mediterranean basin. Phlebotomine sand fly transmits the parasitic agents by inoculation of the flagellate promastigotes in to mammalian host, where they enter macrophages differentiating and multiplying into immobile amastigotes.[2] Visceral Leishmaniasis[3] or Kala Azar is most common pathological form of the disease and caused by *L. donovani*. From the socioeconomic point of view, Leishmaniases are the second most important among all the parasitic diseases.[4] More than 12 million people around the world are affected by Leishmaniasis, with 1.5–2 million new cases and 51000 deaths reported each year.[5]

Chemotherapy of patients with Leishmaniasis is still a serious problem as the treatment options are very limited. Pentavalent antimonial compounds were widely used as primary therapy for 50 years but resistance has developed to them to such an extent in India that they can no longer be used in some regions.[6] Antimonials may also cause acute pancreatitis and cardiac arrhythmia.[7] Only recently amphotericin B, pentamidine, and miltefosin have been discovered as effective antileishmanial drugs. All these drugs also suffer from serious side effects associated with them. Major drawbacks associated with amphotericin B are, it's prohibitively high cost, life threatening first dose anaphylaxis, nephrotoxicity and hypokalemicity.[8] Pentamidine is orally inactive and may show renal, hepatic and pancreatic toxicity along with hypotension and dysglycemia.[9] Miltefosine, originally developed as anticancer drug, is the first orally active antileishmanial agent, which have good efficacy against both visceral as well as cutaneous leishmaniases but suffers from low therapeutic index, teratogenicity in animals, extremely long half life (6-8 days), and relative low efficacy in HIV coinfected patients.[10]

In the light of above facts, there is an urgent need for the development of more efficient, inexpensive, nontoxic, and innovative drugs based on new molecular scaffold for the treatment of leishmaniases. In 1998, a tetrahydro-β-carboline alkaloid buchtienine (**1**)[11] was isolated from *Kopsia griffithii* and found to have good antileishmanial activity ($0.30 > IC_{50} > 1.56$ μg/mL) against *L. donovani*. (Figure 1) A

few years later, antileishmanial activity of harmine (**2)** (Figure 1), a β-carboline amine alkaloid[12] isolated from *Peganum harmala*, was also reported. Pinheiro's group isolated the pyrimidine-β-carboline alkaloid annomontine (**3)** (Figure 1) from the bark of a Brazilian tree *Annona foetida* having antileishmanial activity against *L. braziliensis* with IC_{50} value of 34.8±1.5 μg/mL.[13]



Buchtienine (**1**) Harmine **(2)** Annomontine (**3**)

Figure 1. Natural β-carboline alkaloids with antileishmanial activity

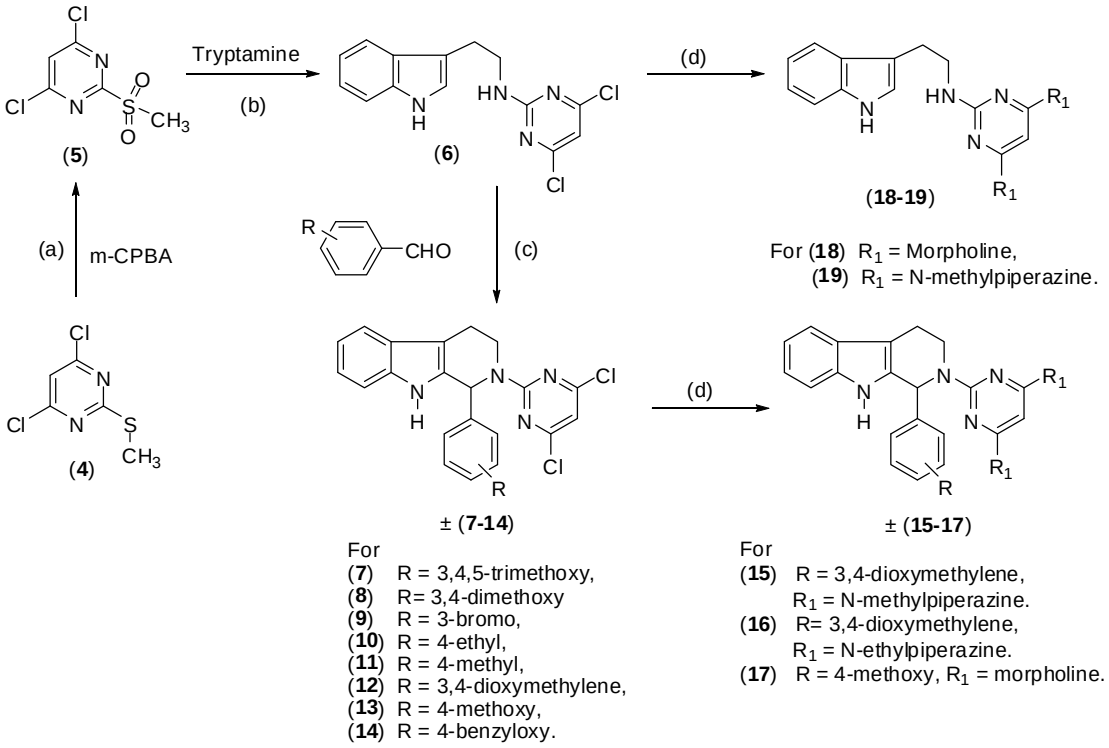
In addition, synthetic 2-aminopyrimidines with hydrophobic handle at 4-position has also been reported as good antileishmanial agents.[14] Prompted by this and in continuation of our efforts toward design and synthesis of novel nitrogen heterocylces as antileishmanial agent,[15] a series of 2-(pyrimidin-2-yl)-1-phenyl-2,3,4,9-tetrahydro-1*H*-β-carbolines has been synthesized and evaluated for antileishmanial activity against *Leishmania donovani*.

3.2 Chemistry:

The synthetic strategy followed for synthesis of 2-(pyrimidin-2-yl)-1-phenyl-2,3,4,9-tetrahydro-1*H*-β-carbolines (**7-19)** is depicted in Scheme 1. It involves oxidation of 2-thiomethyl-4,6-dichloropyrimidine **4** with *m*-chloroperbenzoic acid to its sulfone derivative **5**. Nucleophilic substitution of methane sulfonyl group of **5** with tryptamine furnished *N*-(2-(1*H*-indol-3-yl)ethyl)-4,6-dichloropyrimidin-2-amine **6** in good yield. Pictet Spengler cyclization[16] of **6** with variously substituted benzaldehydes under acidic conditions gave 2-(4,6-dichloropyrimidin-2-yl)-1-phenyl-2,3,4,9-tetrahydro-1*H*-β-carboline derivatives (**7-14)** in high yields. 2-(4,6-diaminopyrimidin-2-yl)-1-phenyl-2,3,4,9-tetrahydro-1*H*-β-carboline derivatives (**15-**

17) and (4,6-diaminopyrimidin-2-yl)-[2-(1*H*-indol-3-yl)-ethyl]-amines (**18-19**) were obtained in good to excellent yields by treating the corresponding dichloro derivatives **12, 13** and **6** with amine nucleophiles. All the tetrahydro-β-carboline derivatives (**7-17**) were obtained as racemic mixtures.

Scheme 1



Reagents and conditions: (a) DCM, 0ºC to rt, 4h; (b) Ethanol, reflux, 2h; (c) PTSA, ethanol, reflux, 3h; (d) Neat Amine, reflux, 10h.

3.3 Results and Discussion:

In vitro antileishmanial evaluation of the 2-(4,6-dichloropyrimidin-2-yl)-1-phenyl-2,3,4,9-tetrahydro-1*H*-β-carboline derivatives (**7-14**), 2-(4,6-diaminopyrimidin-2-yl)-1-phenyl-2,3,4,9-tetrahydro-1*H*-β-carbolines (**15-17**) and 2,4,6-triaminopyrimidines (**18-19**) showed encouraging results as displayed in Table 1. Among the synthesized compounds (**7-19**), all exhibited more than 80% inhibition against promastigotes at 10 µg/mL with the exception of **10**, **12** and **14,** which showed 60.24, 33.57 and 36.85 % inhibition, respectively. *N*-(2-(1*H*-indol-3-yl)ethyl)-4,6-dichloropyrimidin-2-amine **6** was also screened and found to be exhibit 99.78% inhibition against promastigotes,

but against amastigotes it showed poor activity with IC_{50} value of 7.34 µg/mL and selectivity index of 2.80.

Interestingly, the derivative **8** having 3,4-dimethoxyphenyl group at C-1 of tetrahydro-β-carboline ring system, exhibited IC_{50} value of 1.93 µg/ml against amastigotes and selectivity index of 15.43. Both selectivity index and IC_{50} value of **8**

Table 1. *In vitro* antileishmanial acitivity of compounds (**6-19**)

Compound No.	In-Vitro Screening		Cytotoxicity CC_{50} (µg/mL)	Selectivity Index[a] (SI)
	Anti-Promastigote Activity (% inhibition at 10 µg/mL)	Anti-amastigote activity MQ/amast. model IC_{50} (µg/mL)		
6	99.78	7.34	20.59	2.80
7	81.68	3.81	ND	N/A
8	93.06	1.93	29.78	15.43
9	79.65	7.79	14.03	1.81
10	60.24	4.46	30.66	6.87
11	84.15	6.00	24.59	4.09
12	33.57	NA	ND	N/A
13	81.68	3.57	ND	N/A
14	36.85	NA	ND	N/A
15	99.96	0.49	2.80	5.71
16	99.94	0.48	2.50	5.20
17	97.25	4.48	67.67	15.10
18	99.72	3.05	19.35	6.34
19	100	1.74	18.37	10.55
SSG	40-50*	53.62	297.38	6.38
Pentamidine	100	12.11	25.15	2.07

NA: not active. ND: not determined. [a]Selectivity index (SI) defined by the ratio CC_{50} (J774A.1 cells)/IC_{50} (Leishmania amastigotes). SSG: sodium stilbogluconate. * SSG shows 40-50% inhibition against promastigotes at 500 µg/mL. N/A: not applicable.

were much better than that of reference drugs sodium stilbogluconate (SSG) and pentamidine. It can be observed in Table 1 that antileishmanial activity is dependent on nature and position of substituents on aryl ring at C-1 of tetrahydro-β-carboline ring system. In comparison to **8**, presence of one more methoxy group as in **7** or one less methoxy group as in **13** led to two folds decrease in activity against amastigotes. Compounds **7** and **13** were highly toxic against J774A.1 cells, so were not selective against amastigotes. 3-bromophenyl substituent in compound (**9**) was also found to be detrimental to antiamastigote activity. Higher IC_{50} values and lower selectivity indices for compounds **10** and **11** indicated that 4-methyl or ethyl substituents were not good for antiamastigote activity. 3,4-Dioxymethylenephenyl and benzyloxyphenyl derivatives **12** and **14** were inactive against amastigotes. It indicates that 3,4-dimethoxyphenyl substituent at C-1 of tetrahydro-β-carboline ring system is necessary for the antileishmanial activity.

Amongst 2-(4,6-diaminopyrimidin-2-yl)-1-phenyl-2,3,4,9-tetrahydro-1*H*-β-carboline derivatives, antiamastigote activities of compounds **15** and **16** were good, but they didn't have selectivity indices comparable to that of **8**. Inhibitory potency of compound **17** against amastigotes was less, but value of selectivity index was comparable to compound **8**. Compound **18** also inhibited amastigotes with IC_{50} value of 3.05μg/mL and poor selectivity index. Similarly, compound **19** was as effective against amastigotes as compound **8** but has lower selectivity index.

Compound **8** was tested for *in vivo* potency with a dose of 50mg/Kg, (ip) for 5 days in golden hamsters (*Mesocricetus auretus*) infected with MHOM/IN/80/Dd_8 strain of *L. donovani*. It has shown above moderate percentage inhibition of 56.58.

Table 2. *In vivo* antileishmanial activity of compound **8**

Comp. No	Dosage route[a]	Dosage	% Parasitemia inhibition[b]
8	ip	5×50 mg/kg	59.52
Pentamidine®	ip	5×20 mg/kg	84.10
SSG®	ip	5×40 mg/kg	92

[a] ip: intra peritoneal. [b]The *in-vivo* leishmanicidal activity was determined in golden hamsters (*Mesocricetus auretus*) infected with MHOM/IN/80/Dd_8 strain of *L. donovani*.

Structure activity relationship reveals that 3,4-dimethoxyphenyl substituent at C-1 of tetrahydro-β-carboline ring system in 2-(4,6-dichloropyrimidin-2-yl)-1-phenyl-2,3,4,9-tetrahydro-1*H*-β-carboline derivatives increase the antileishmanial activity and these results will be used as basis strategy point for the design of new molecules with improved antileishmanial activity.

Effectiveness of certain molecules as both antiprotozoal and anticancer agents,[17] antileishmanial and anticancer activities of tetrahydro-β-carboline derivatives[18] led us to screen all the synthesized compounds **6-19** for their cytotoxicity against a panel of human cancer cell lines. Only compound **15** showed moderate cytotoxicity against C33A, MCF7, DU145, and KB cancer cell lines with IC_{50} values of 1.1, 1.2, 1.5, and 2.0 μg/mL, respectively.(Table 3.)

Table 3. *In vitro* anticancer activity of compounds (**9-19**).

Compound	IC_{50} (μg/mL)				
No.	**DU 145**	**MCF7**	**C33A**	**KB**	**VERO**
9	15.8	23.4	21.7	15.6	24.3
10	23.0	32.4	19.8	22.8	37.8
11	20.2	28.0	24.9	21.6	33.7
12	8.9	4.3	3.3	3.9	8.2
13	10.1	5.2	4.2	4.5	9.8
15	1.5	1.2	1.1	2.0	2.6
16	6.2	3.6	4.1	4.5	5.9
17	4.5	3.9	2.5	5.1	7.8
19	21.3	35.2	26	33.4	29.7

3.4 Conclusion:

In summary, synthesis and biological evaluation of the series 2-(pyrimidin-2-yl)-1-phenyl-2,3,4,9-tetrahydro-1*H*-β-carboline derivatives led us to discovery of compound **8** as good antileishmanial agent which is more active than SSG and pentamidine *in vitro*. Selectivity index of compound **8** is 2.4, and 7.4 folds higher than SSG and pentamidine, respectively. These preliminary investigations revealed

that 2-(pyrimidin-2-yl)-1-phenyl-2,3,4,9-tetrahydro-1*H*-β-carboline class can be served as prototype for development of more efficacious antileishmanial agents.

3.5 Experimental:

The melting points were recorded on an electrically heated melting point apparatus and are uncorrected. IR spectra were recorded on Beckman Aculab-10, Perkin Elmer 881 and FTIR Shimadzu 8201PC spectrophotometers either on KBr discs or in neat. Nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance DRX-300 MHz FT spectrometer using TMS as an internal reference. Mass spectra were recorded on JEOL SX 102/DA 6000 mass spectrometer using Argon/Xenon (6 kV, 10 mA). Chemical analysis was carried out on Carlo-Erba-1108 instrument.

Typical procedure for the synthesis of compound (6):

To a solution of 2-thiomethyl-3,5-dichloropyrimidine **4** (1 eq.) in dry DCM was added m-chloroperbenzoic acid (3 eq.) at 0 °C and stirred for 3h with gradual rise in temperature to room temperature. Then, reaction was quenched by saturation solution of sodium bicarbonate and diluted with DCM. Organic phase was separated, dried by Na_2SO_4 and evaporated *in vacuo*. White solid so obtained was refluxed with equivalent amount of tryptamine in ethanol for 2 hours. After TLC analysis showed completion of the reaction, ethanol was evaporated at reduced pressure and purified by column chromatography to obtain pure compound **6**.

***N*-(2-(1*H*-indol-3-yl)ethyl)-4,6-dichloropyrimidin-2-amine (6):**

Yield: 68%, mp 132-134ºC; IR (KBr): 3692, 3477, 3019, 2928, 2857, 1573, 1522, 1430, 1216, 1093, 1044 cm^{-1}; ^1H NMR ($CDCl_3$, 300MHz): δ (ppm) 8.00 (s, 1H), 7.66 (d, 1H, J = 8.1Hz), 7.37 (d, 1H, J = 9.6Hz), 7.16 (t, 1H, J = 7.8Hz), 7.22 (t, 1H, J = 6.9Hz), 7.04 (s, 1H), 6.59 (s, 1H), 5.67 (s, 1H), 3.78 (q, 2H, J = 6.6Hz), 3.08 (t, 2H, J = 6.9Hz); ^{13}C NMR($CDCl_3$, 75MHz): 161.57, 136.46, 127.24, 122.27, 122.10, 119.62, 119.55, 118.81, 112.61, 111.23, 108.72, 96.18, 41.73, 25.08. Anal. Calcd. for $C_{14}H_{12}Cl_2N_4$: C 54.74, H 3.94, N 18.24; Found: C 54.37, H 3.56, N 17.93 %.

General procedure for the synthesis of compounds (7-14):

The mixture of compound **6** (1 eq.) and substituted benzaldehyde (1 eq.) was refluxed in ethanol in presence of catalytic amount of PTSA for 3 hours. After

completion of reaction, solvent was removed *in vacuo* and resulting solid residue was column chromatographed to afford respective compounds yielding in range of 65-75%.

2-(4,6-Dichloropyrimidin-2-yl)-1-(3,4,5-trimethoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline (7):

Yield: 75%; mp: 198-200ºC; IR (KBr): 3289, 3101, 2994, 2937, 2843, 1579, 1509, 1457, 1350, 1324, 1298, 1247, 1183, 1120 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz): δ (ppm) 8.05 (bs, 1H), 7.57 (d, 1H, J = 7.5Hz), 7.31 (d, 1H, J = 7.5 Hz), 7.20- 7.13 (m, 2H), 6.91 (s, 1H), 6.71 (s, 2H), 6.58 (s, 1H), 5.07 (dd, 1H, J =13.2, 3.3Hz), 3.85 (s, 3H), 3.77 (s, 6H), 3.49-3.39 (m, 1H), 3.09-2.92 (m, 2H); ^{13}C ($CDCl_3$, 75MHz): 163.9, 160.30, 152.91, 137.58, 136.56, 135.19, 131.44, 126.57, 122.08, 119.58, 118.29, 111.08, 110.60, 108.05, 105.99, 60.61, 55.97, 54.64, 38.64, 21.24. Anal. Calcd. for $C_{24}H_{22}Cl_2N_4O_2$: C 59.39, H 4.57, N 11.54; Found: C 59.02, H 4.28, N 11.33 %.

2-(4,6-Dichloropyrimidin-2-yl)-1-(3,4-dimethoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline (8):

Yield: 72%, mp 209-211ºC; IR (KBr): 3371, 3105, 3007, 2945, 2838, 1682, 1581, 1512, 1459, 1384, 1346, 1304, 1269, 1245, 1136 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz): δ (ppm) 7.81 (s, 1H), 7.59 (d, 1H, J = 7.5Hz), 7.33-7.14 (m, 4H), 7.16 (s, 1H), 6.84-6.74 (m, 2H), 6.58 (s, 1H), 5.01 (dd, 1H, J = 13.4, 4.2Hz), 3.86 (s, 3H), 3.85 (s, 3H), 3.49-3.40 (m, 1H), 3.12- 2.96 (m, 2H); ^{13}C NMR ($CDCl_3$, 75MHz): 160.30, 148.95, 148.81, 136.38, 132.07, 131.76, 126.65, 122.17, 120.91, 119.61, 118.33, 112.32, 111.03, 110.76, 110.59, 108.04, 55.84, 55.78, 54.13, 38.21, 21.14. Anal. Calcd. for $C_{23}H_{20}Cl_2N_4O_2$: C 60.67, H 4.43, N 12.30; Found: C 60.48, H 4.14, N 12.11 %.

1-(3-Bromophenyl)-2-(4,6-dichloropyrimidin-2-yl)-2,3,4,9-tetrahydro-1*H*-β-carboline (9):

Yield: 65%; mp: 158-160ºC; IR (KBr): 3686, 3459, 3019, 1727, 1569, 1510, 1445, 1375, 1216 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz): δ (ppm) 7.59 (m, 2H), 7.45 (d, 1H, J = 7.8Hz), 7.39-7.32 (m, 2H), 7.25-7.14 (m, 3H), 7.03 (s, 1H), 6.61 (s, 1H), 4.94 (dd, 1H, J = 13.2, 4.5Hz), 3.46-3.34 (m, 1H), 3.15-2.86 (m, 2H); ^{13}C ($CDCl_3$, 75MHz): 160.35, 141.85, 136.39, 131.57, 131.54, 130.68, 130.19, 127.29, 126.63, 122.81 122.50,

119.89, 118.48, 111.16, 111.13, 108.63, 53.78, 38.33, 20.94. Anal. Calcd. for $C_{21}H_{15}BrCl_2N_4$: C 53.19, H 3.19, N 11.82; Found: C 52.84, H 2.92, N 11.66 %.

2-(4,6-Dichloropyrimidin-2-yl)-1-(4-ethylphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline (10):

Yield: 72%; mp: 187-189ºC; IR (KBr): 3396, 3123, 3055, 2926, 1581, 1509, 1436, 1372, 1303, 1237, 1188 cm^{-1}; ^1H NMR ($CDCl_3$, 300MHz): δ (ppm) 7.73 (s, 1H), 7.58 (d, 1H, J = 7.5Hz), 7.36-7.29 (m, 2H), 7.23-7.15 (m, 4H), 7.06 (s, 1H), 6.57 (s, 1H), 4.98 (dd, 1H, J = 13.2, 4.5 Hz), 3.49-3.35 (m, 1H), 3.12-2.93 (m, 2H), 2.64 (q, 2H, J = 7.8Hz), 1.23 (t, 3H, J = 7.8Hz); ^{13}C ($CDCl_3$, 75MHz): 160.37, 144.57, 136.85, 136.31, 131.84, 128.75, 128.08, 126.74, 122.20, 119.72, 118.35, 111.01, 110.72, 108.12, 54.02, 38.19, 28.57, 21.08, 15.51. Anal. Calcd. for $C_{23}H_{20}Cl_2N_4$: C 65.25, H 4.76, N 13.23; Found: C 64.95, H 4.52, N 12.97 %.

2-(4,6-Dichloropyrimidin-2-yl)-1-p-tolyl-2,3,4,9-tetrahydro-1*H*-β-carboline (11) :

Yield: 70%; mp: 216-218ºC; IR (KBr): 3691, 3401, 3020, 1728, 1569, 1510, 1445, 1372, 1217 cm^{-1}; ^1H NMR ($CDCl_3$, 300MHz): δ (ppm) 7.70 (s, 1H), 7.58 (d, 1H, J = 7.5Hz), 7.31-7.25 (m, 3H), 7.24-7.11 (m, 4H), 7.05 (s, 1H), 6.57 (s, 1H), 4.98 (dd, 1H, J = 13.2, 4.2Hz), 3.43-3.35 (m, 1H), 3.03-2.94 (m, 2H), 2.33 (s, 3H); ^{13}C ($CDCl_3$, 75MHz): 161.92, 160.35, 138.23, 136.61, 136.31, 131.81, 129.26, 128.64, 126.72, 122.19, 119.70, 118.34, 110.99, 110.72, 108.12, 54.01, 38.19, 21.16, 21.06. Anal. Calcd. for $C_{22}H_{18}Cl_2N_4$: C 64.56, H 4.43, N 13.69; Found: C 64.36, H 4.15, N 13.43 %.

1-(Benzo[d][1,3]dioxol-5-yl)-2-(4,6-dichloropyrimidin-2-yl)-2,3,4,9-tetrahydro-1*H*-β-carboline (12) :

Yield: 68%; mp: 173-175ºC; IR (KBr): 3404, 2922, 1572, 1509, 1435, 1304, 1240 cm^{-1}; ^1H NMR ($CDCl_3$, 300MHz): δ (ppm) 7.73 (bs, 1H), 7.55 (d, 1H, J = 7.5Hz), 7.30 (d, 1H, J = 7.8Hz), 7.22-7.11 (m, 2H), 6.99-6.93 (m, 2H), 6.88 (d, 1H, J = 8.1Hz), 6.73 (d, 1H, J = 7.8Hz), 6.56 (s, 1H), 5.93 (d, 2H, J = 1.8Hz), 4.95 (dd, 1H, J = 13.2, 4.8Hz), 3.41-3.32 (m, 1H), 2.99-2.85 (m, 2H); ^{13}C ($CDCl_3$+CD_3OD, 75MHz): 164.18, 151.60, 151.28, 140.39, 137.70, 136.73, 135.59, 133.57, 130.36, 126.07, 125.65, 123.01, 121.95, 112.85, 112.28, 111.87, 111.80, 104.98, 57.86, 41.86, 24.82. Anal.

Calcd. for $C_{22}H_{16}Cl_2N_4O_2$: C 60.15, H 3.67, N 12.75; Found: C 59.91, H 3.46, N 12.54 %.

2-(4,6-Dichloro-pyrimidin-2-yl)-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline (13):

Yield: 69%; mp: 191-193ºC; IR (KBr): 3872, 3399, 2930, 2844, 1586, 1511, 1454, 1369, 1304, 1249, 1178 cm^{-1} ; ^1H NMR ($CDCl_3$, 300MHz): δ (ppm) 7.43 (d, 1H, J = 7.5Hz), 7.23-7.17 (m, 3H), 7.07-6.97 (m, 2H), 6.90 (s, 1H), 6.70 (d, 2H, J = 8.4Hz), 6.44 (s, 1H), 4.80 (dd, 1H, J = 13.2, 4.8Hz), 3.65 (s, 3H), 3.28-3.18 (m, 1H), 2.88-2.74 (m, 2H); ^{13}C ($CDCl_3$+CD_3OD, 75MHz): 164.78, 163.65, 162.73, 139.76, 135.45, 135.29, 133.21, 129.90, 125.14, 122.58, 121.46, 117.06, 114.44, 113.33, 111.28, 58.58, 57.06, 41.37, 24.36. Anal. Calcd. for $C_{22}H_{18}Cl_2N_4O$: C 62.13, H 4.27, N 13.17; Found: C 61.83, H 4.02, N 12.93 %.

1-(4-(Benzyloxy)phenyl)-2-(4,6-dichloropyrimidin-2-yl)-2,3,4,9-tetrahydro-1*H*-β-carboline (14):

Yield: 74%; mp: 184-186ºC; IR (KBr): 3407, 2924, 2845, 1571, 1508, 1446, 1238, 1172 cm^{-1} ; ^1H NMR ($CDCl_3$, 300MHz): δ (ppm) 7.68 (s, 1H), 7.55 (d, 1H, J = 7.2Hz), 7.42-7.29 (m, 8H), 7.21-7.11 (m, 2H), 7.02 (s, 1H), 6.90 (d, 2H, J = 8.7Hz), 6.55 (s, 1H), 5.03 (s, 2H), 4.94 (dd, 1H, J = 13.8, 4.5Hz), 3.38-3.31 (m, 1H), 2.99-2.91 (m, 2H); ^{13}C ($CDCl_3$, 75MHz): 160.30, 158.81, 136.82, 136.28, 132.01, 131.84, 130.00, 128.64, 128.06, 127.46, 126.71, 122.21, 119.72, 118.35, 114.82, 111.00, 110.75, 108.11, 70.03, 53.66, 38.04, 21.07. Anal. Calcd. for $C_{28}H_{22}Cl_2N_4O$: C 67.07, H 4.42, N 11.17; Found: C 66.83, H 4.18, N 10.98 %.

General procedure for the synthesis of compounds (15-19)

To synthesize 2,4,6-triaminopyrimidine derivatives (**15-19)** corresponding dichloro derivative were refluxed in neat amine for 10 hours. After completion of the reaction, amine was evaporated *in vacuo* and resulting solid was dissolved in $CHCl_3$, washed with 2% HCl in water and purified by column chromatography to afford pure compounds with yields ranging from 73-85%.

1-(Benzo[d][1,3]dioxol-5-yl)-2-(4,6-bis(4-methylpiperazin-1-yl)pyrimidin-2-yl)-2,3,4,9-tetrahydro-1*H*-β-carboline (15) :

Yield: 73%; mp: 204-206ºC; ESMS: 567 (M+1); IR (KBr): 3783, 3689, 3619, 3413, 3020, 2927, 2361, 1561, 1426, 1371, 1216 cm^{-1}; ^1H NMR ($CDCl_3$, 300MHz): δ (ppm) 8.74 (s, 1H), 7.55 (d, 1H, J = 7.2Hz), 7.27 (d, 1H, J = 6.23Hz), 7.17-7.11 (m, 3H), 7.01 (s, 1H), 6.82 (d, 1H, J = 7.8Hz), 6.68 (d, 1H, J = 7.8Hz), 5.89-5.87 (m, 2H), 5.12 (s, 1H), 4.97 (dd, 1H, J = 13.2, 4.3Hz), 3.61(bs, 8H), 3.19-3.11 (m, 1H), 2.99-2.89 (m, 1H), 2.79 (dd, 1H, J = 14.7, 2.7 Hz), 2.50 (bs, 8H), 2.31 (s, 6H); ^{13}C ($CDCl_3$, 75MHz): 164.53, 160.35, 147.64, 146.87, 136.19, 136.12, 133.21, 127.22, 121.72, 121.50, 119.13, 118.23, 111.16, 110.72, 109.08, 107.69, 100.90, 73.04, 54.82, 52.86, 46.11, 44.20, 37.06, 21.14. Anal. Calcd. for $C_{32}H_{38}N_8O_2$: C 67.82, H 6.76, N 19.77; Found: C 67.66, H 6.39, N 19.52 %.

1-(Benzo[d][1,3]dioxol-5-yl)-2-(4,6-bis(4-ethylpiperazin-1-yl)pyrimidin-2-yl)-2,3,4,9-tetrahydro-1*H*-β-carboline (16) :

Yield: 81%; mp: 179-181ºC; ESMS: 595 (M+1); IR (KBr): 3693, 3463, 3019, 2927, 2848, 1560, 1427, 1350, 1304, 1216, 1166, 1124 cm^{-1}; ^1H NMR ($CDCl_3$, 300MHz): δ (ppm) 8.65 (s, 1H), 7.55 (d, 1H, J = 7.2Hz), 7.27 (d, 1H, J = 6.24 Hz), 7.19-7.09 (m, 3H), 7.01 (s, 1H), 6.83 (m, 1H), 6.68 (d, 1H, J = 8.1Hz), 5.91-5.89 (m, 2H), 5.12 (s, 1H), 4.98 (dd, 1H, J = 13.2, 4.8Hz), 3.62 (bs, 8H), 3.22-3.14 (m, 1H), 2.95-2.92 (m, 1H), 2.75 (dd, 1H, J = 15.0, 3.0 Hz), 2.55 (bs, 8Hz), 2.44 (q, 4H, J = 6.9 Hz), 0.91 (t, 6H, J = 6.9Hz); ^{13}C ($CDCl_3$, 75MHz): 164.50, 160.32, 147.61, 146.86, 136.19, 136.15, 133.21, 127.22, 121.79, 121.44, 119.06, 118.23, 111.10, 110.69, 109.14, 107.70, 100.89, 72.95, 52.84, 52.57, 52.37, 44.19, 36.97, 21.16, 11.76. Anal. Calcd. for $C_{34}H_{42}N_8O_2$: C 68.66, H 7.12, N 18.84; Found: C 68.34, H 6.92, N 18.69 %.

2-(4,6-Dimorpholin-4-yl-pyrimidin-2-yl)-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline (17) :

Yield: 76%; mp: 145-147ºC; ESMS: 527 (M+1); IR (KBr): 3781, 3692, 3405, 3020, 2923, 2852, 1560, 1509, 1421, 1368, 1300, 1216 cm^{-1}; ^1H NMR ($CDCl_3$, 300MHz): δ (ppm) 10.85 (s, 1H), 7.44 (d, 1H, J = 7.8Hz), 7.28 (d, 1H, J = 8.1Hz), 7.22 (d, 2H, J = 8.4Hz), 7.09-6.96 (m, 3H), 6.88 (d, 2H, J = 8.7Hz), 5.40 (s, 1H), 4.82 (dd, 1H, J = 12.6, 3.9Hz), 3.76 (s, 3H), 3.71 (bs, 8H), 3.52 (bs, 8H), 3.18-3.06 (m, 1H), 2.81-2.69 (m, 2H); ^{13}C (DMSO-d_6, 75MHz): 164.81, 160.31, 158.97, 136.60, 134.49, 134.22, 129.45, 126.99, 121.33, 118.84, 118.16, 114.03, 111.48, 109.16, 73.79, 66.43, 55.53,

52.74, 44.94, 37.16, 21.10. Anal. Calcd. for $C_{30}H_{34}N_6O_3$: C 68.42, H 6.51, N 15.96; Found: C 68.08, H 6.25, N 15.62 %.

(4,6-Dimorpholin-4-yl-pyrimidin-2-yl)-[2-(1H-indol-3-yl)ethyl]amine (18) :

Yield: 82%; mp: 190-192ºC; ESMS: 409 (M+1); IR (KBr): 3695, 3395, 3355, 3046, 2956, 2921, 2850, 1722, 1579, 1549, 1460, 1439, 1360, 1333, 1299, 1259, 1222, 1186 cm^{-1}; ^1H NMR ($CDCl_3$, 300MHz): δ (ppm) 8.08 (bs, 1H), 7.66 (d, 1H, J = 7.8Hz), 7.38 (d, 1H, J = 7.8Hz), 7.24-7.10 (m, 2H), 7.05 (s, 1H), 5.11 (s, 1H), 3.77 (t, 8H, J = 4.5Hz), 3.71 (t, 2H, J = 6.6Hz), 3.51 (t, 8H, J = 4.5Hz), 3.05 (t, 2H, J = 6.6Hz); ^{13}C ($CDCl_3$+CD_3OD, 75MHz): 168.80, 165.57, 140.35, 131.35, 126.20, 126.04, 125.52, 122.71, 122.57, 117.04, 115.11, 70.57, 48.69, 45.60, 29.65. Anal. Calcd. for $C_{22}H_{28}N_6O_2$: C 64.68, H 6.91, N 20.57; Found: C 64.53, H 6.76, N 20.23 %.

[4,6-Bis(4-methylpiperazin-1-yl)pyrimidin-2-yl][2-(1H-indol-3-yl)ethyl]amine (19) :

Yield: 85%; mp 162-164ºC; ESMS : 435 (M+1); IR (KBr): 3781, 3691, 3475, 3019, 2927, 2852, 2805, 1573, 1445, 1364, 1281, 1216 cm^{-1}; ^1H NMR ($CDCl_3$, 300MHz): δ (ppm) 8.19 (bs, 1H), 7.66 (d, 1H, J = 7.8Hz), 7.36 (d, 1H, J = 7.8Hz), 7.23-7.08 (m, 2H), 7.05 (s, 1H), 5.12 (s, 1H), 4.83 (bs, 1H), 3.74-3.68 (m, 2H), 3.57 (t, 8H, J = 4.8Hz), 3.05 (t, 2H, J = 6.9Hz), 2.47 (t, 8H, J = 4.8Hz), 2.33 (s, 6H); ^{13}C NMR ($CDCl_3$, 75MHz): 164.65, 161.66, 136.41, 127.60, 121.99, 121.89, 119.13, 118.95, 113.90, 111.13, 73.48, 54.83, 46.17, 44.28, 41.78, 29.69. Anal. Calcd. for $C_{24}H_{34}N_8$: C 66.33, H 7.89, N 25.78; Found: C 66.07, H 7.58, N 25.42 %.

3.6 Antileishmanial activity:

3.6.1 Antipromastigote activity

The *Leishmania donovani* promastigotes (MHOM/IN/80/Dd8; originally obtained from Imperial College, London) were transfected with firefly luciferase gene and the transfectants were maintained in medium 199 (Sigma Chemical Co., USA) supplemented with 10% foetal calf serum (GIBCO) and 1% penicillin (50 U/mL), streptomycin (50 μg/mL) solution (Sigma) under pressure of G418 (Sigma). The *in vitro* effect of the compounds on the growth of promastigotes was assessed by monitoring the luciferase activity of viable cells after treatment. The transgenic

promastigotes of late log phase were seeded in 5×10^5/100 µl medium 199 in 96-well flat-bottomed microtiter (MT) plates (CELLSTAR) and incubated for 72 h in medium alone or in the presence of serial dilutions of drugs (1–10 µg/mL) in DMSO.[19] Parallel dilutions of DMSO were used as controls. After incubation, an aliquot (50 µl) of promastigote suspensionwas aspirated from each well of a 96-well plate and mixed with an equal volume of Steady Glo® reagent (Promega) and luminescence was measured by a luminometer. The values were expressed as relative luminescence unit (RLU). The inhibition of parasitic growth is determined by comparison of the luciferase activity of drug treated parasites with that of untreated controls by the general formula:

Percentage inhibition = (N – n × 100)/N

where N is average relative luminescence unit (RLU) of control wells and n is average RLU of treated wells.

3.6.2 Antiamastigote activity

For assessing the activity of compounds against the amastigote stage of the parasite, mouse macrophage cell line (J774A.1) infected with promastigotes expressing luciferase firefly reporter gene was used. Cells were seeded in a 96-well plate (1.5×10^4 cells/100 µL/well) in RPMI-1640 containing 10% foetal calf serum and the plates were incubated at 37 °C in a CO_2 incubator. After 24h, the medium was replaced with fresh medium containing stationary phase promastigotes (2.25×10^5/100 µL/well). Promastigotes invade the macrophage and are transformed into amastigotes. The test material in appropriate concentrations (0.25–10 µg/mL) in complete medium was added after replacing the previous medium and the plates were incubated at 37 °C in a CO_2 incubator for 72h. After incubation, the drug containing medium was decanted and 50 µL PBS was added in each well and mixed with an equal volume of Steady Glo® reagent. After gentle shaking for 1–2 min, the reading was taken in a luminometer.[17] The inhibition of parasitic growth is determined by comparison of the luciferase activity of drug treated parasites with that of untreated controls as described above. IC_{50} of antileishmanial activity were evaluated by logit regression analysis.

3.6.3 Cytotoxicity assay:

The cell viability was determined using the MTT assay. J774A.1 cell line was maintained in RPMI medium (Sigma), supplemented with 10% foetal calf serum and 40 mg/mL gentamycin. Exponentially growing cells (1×10^4 cells/100 µL/well) were incubated with different drug concentrations for 72 h and were incubated at 37 °C in a humidified mixture of CO_2 and 95% air in an incubator. Stock solutions of compounds were initially dissolved in DMSO and further diluted with fresh complete medium. After incubation, 25 µL of MTT reagent (5 mg/mL) in PBS medium, followed by syringe filtration were added to each well and incubated at 37 °C for 2h. At the end of the incubation period, the supernatant was removed by tilting plate completely without disturbing cell layer and 150 µL of pure DMSO are added to each well. After 15 min of shaking the readings were recorded as absorbance at 544 nm on a microplate reader. The cytotoxic effect was expressed as 50% lethal dose, i.e., as the concentration of a compound which provoked a 50% reduction in cell viability compared to cell in culture medium alone. IC_{50} values were estimated through the preformed template as described by Huber and Koella.[20]

3.6.4 *In vivo* assay:

The *in vivo* leishmanicidal activity was determined in golden hamsters (*Mesocricetus auretus*) infected with MHOM/IN/80/Dd_8 strain of *L. donovani* obtained through the courtesy of P.C.C. Garnham, Imperial College, London (U.K.). For *in vivo* evaluation of compounds, the method of Beveridge as modified by Bhatnagar et al.[21] and Gupta et al.[22] was employed. Golden hamsters (of either sex) weighing 40-45 g were infected intracardially with $1x10^7$ amastigotes per animal. Pretreatment spleen biopsy in all the animals was carried out to assess the degree of infection. The animals with +1 infection (5-15 amastigotes/100 spleen cell nuclei) were included in the chemotherapeutic trials. The infected animals are randomized into several groups on the basis of their parasitic burdens. Four to six animals were used for each test sample. Drug treatment by *ip* route is initiated after 2 days of biopsy and continued for 10 consecutive days. Post-treatment biopsies are done on day 7 of the last drug administration and amastigote counts are assessed by Giemsa staining. Intensity of infection in both, treated and untreated animals, as also the initial count in treated

animals is compared and the efficacy is expressed in terms of percentage inhibition (PI) using the following formula:-

PI = 100- (ANAT x 100/INAT x TIUC)

Where PI is Percent Inhibition of Amastigotes Multiplication

ANAT is Actual Number of Amastigotes in Treated animals

INAT is Initial Number of Amastigotes in Treated animals

TIUC is Times Increase of parasites in Untreated Control animals.

3.7 Anticancer Activity: Please see section no. 2.6.1.

3.8 References:

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Chapter 4a

Marine 2-Aminoimidazole, Glycociamidine Alkaloids and Their Synthetic Analogues: New Leads for Drug Development

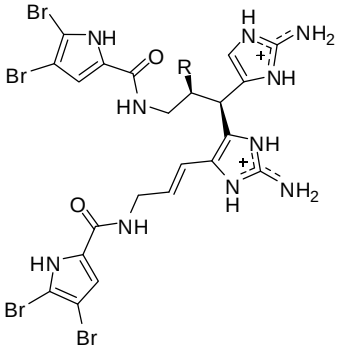
4.1.1 Introduction:

Nature is the richest source of novel compound classes and leads for drug development. In the pregenomic era, more than 80% of drug substances were natural products or inspired by natural products.[1] Natural products based drug discovery has undergone many changes in past 30 years, with noticeable decline in participation by major pharmaceutical industries by the mid nineties and renaissance in past 5 years due to failure of competing technologies like combinatorial chemistry. Significant number of molecules of marine origin or have synthesized taking inspiration from marine natural products are either in or approaching Phase II/III clinical trials in various diseases, clearly demonstrate the increasing interest in marine natural products as leads for drug development.[2] First marine drug, ziconotide[3] (ω-conotoxin MVIIA), isolated from a tropical marine cone snail, was approved in United States in 2004 for the treatment of chronic pain in spinal chord injury, under trade name Prialt. In October 2007, another marine natural product ET-743 (ecteinasidin-743/Yondelis/trabectedin), the antitumour compound from sea-squirt was approved by European Union for the treatment of soft tissue sarcoma. More than 5300[4] different products are known from marine sponges and their associated microorganisms, and more than 200 new metabolites from sponges are reported each year. It demonstrates that potential of marine natural products as leads for drug development has recently being realized. In this chapter efforts are made to present an overview on isolation, structure and biological activities of marine 2-aminoimidazole, glycociamidine alkaloids and their synthetic analogues.

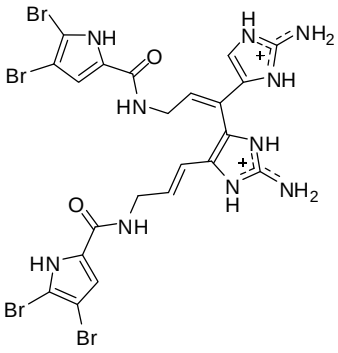
4.1.2 Marine 2-aminoimidazole and glycociamidine alkaloids:

4.1.2.1 Nagelamides:

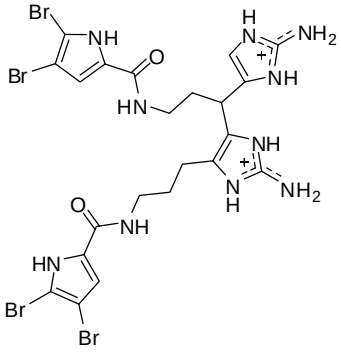
Nagelamides, the dimeric bromopyrrole alkaloids were isolated from Okinawan marine sponge *Agelas* sp. Nagelamide A (**1**), G (**7**), and H (**8**)[5] inhibited a major serine/threonine-protein phosphatase type 2A enzyme with IC_{50} value of 48, 13, and 46 μM, respectively. They also exhibited antibacterial activity against *Micrococcus luteus* (gram positive), *Bacillus subtilis* (gram positive), *Escherichia coli* (gram negative). Among these, **1** was most active against both gram positive and gram negative bacteria with MIC values of 2.08, 16.7 and 33.3 μg/mL against *M. luteus, B.*



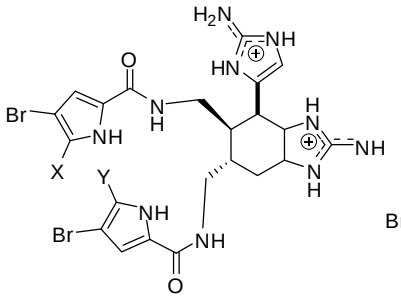
Nagelamide A (**1**): R = H
Nagelamide B (**2**): R = OH



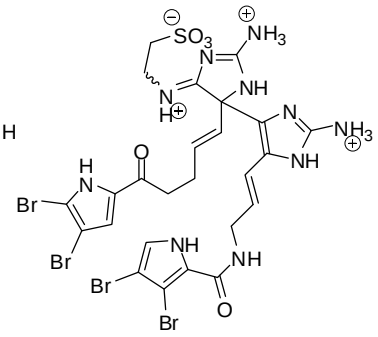
Nagelamide C (**3**)



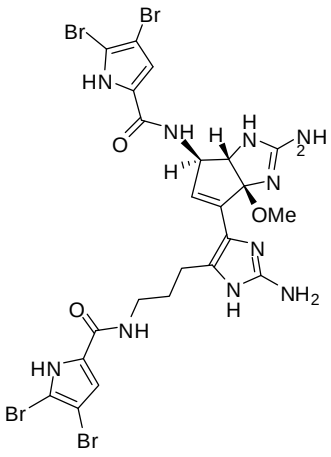
Nagelamide D (**4**)



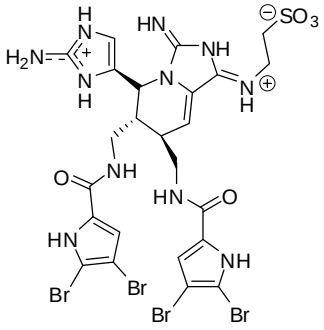
Nagelamide E (**5**): X = Y = H
Nagelamide F (**6**): X = Br, Y = H
Nagelamide G (**7**): X = Y = Br



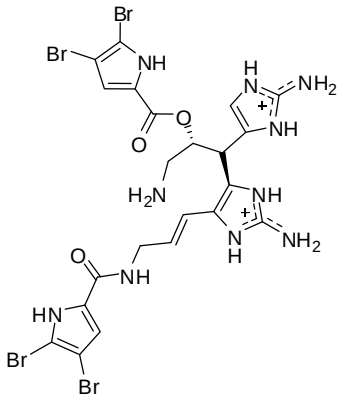
Nagelamide H (**8**)



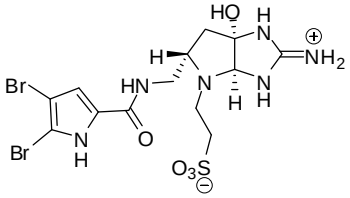
Nagelamide J (**9**)



Nagelamide K (**10**)

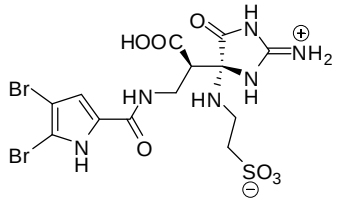


Nagelamide L (**11**)

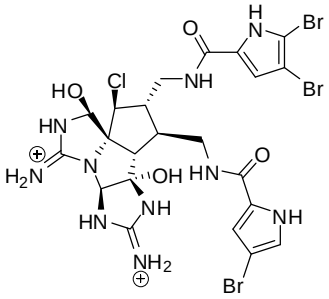


Nagelamide M (**12**)

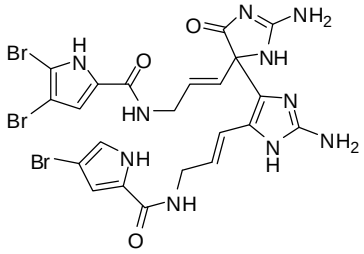
subtilis and *E. coli.,* respectively, while others were less potent with MIC values more than 33.3 µg/mL (Table 1). Nagelamide J (**9**)[6], is the first dimeric bromopyrrole alkaloid possesing a cyclopentane ring fused to an 2-aminoimidazole ring, was isolated from a sample of *Agelas* sp. (SS-1077) collected off Unten-Port, Okinawa. It exhibited antimicrobial activity against *Staphylcoccus aureus* and *Cryptococcus neoformans* with MIC values of 8.35 and 16.7 µg/mL, respectively (Table 1).



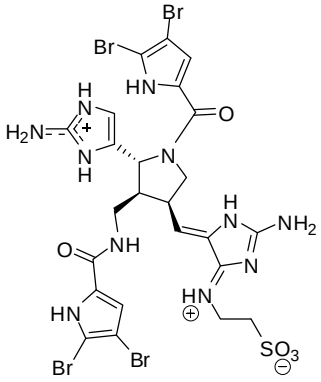
Nagelamide N (**13**)



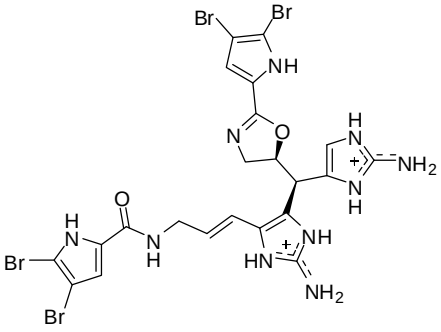
Nagelamide O (**14**)



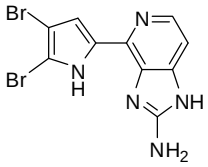
Nagelamide P (**15**)



Nagelamide Q (**16**)



Nagelamide R (**17**)



Agelamide (**18**)

Nagelamide K (**10**)[7] is unique in having piperidinoiminoimidazolone ring with 2-aminoimidazole ring and a taurine unit. Both Nagelamide K (**10**) and L (**11**) are antibacterial agents having activity against *M. luteus* with MIC value of 16.7 µg/mL for each. Nagelamide M (**12**) and N (**13**)[8] both exhibited antifungal activity against *Aspergillus niger* with MIC value of 33.3 µg/mL each (Table 1).

Nagelamide O (**14**) is a rare alkaloid having perhydrocyclopenta-imidazolo-imidazole carbon skeleton. It showed weak antibacterial activity against *B. subtilis*, *M. luteus*, and *S. aureus* with MIC values of 33.3 µg/mL each. Nagelamide P (**15**) was inactive both as antifungal and antibacterial agent.[9] Key structural feature of Nagelamide Q (**16**) is the presence of pyrrolidine ring which separates both 2-aminoimidazole rings.

Alkaloid **16** was found to be active against *B. subtilis, Trichophyton mentagrophytes, Candida albicans, Cryptococcus neoformans,* and *A. niger* with MIC values of 13.0, 6.0, 13.0, 13.0 and 13.0 μg/mL. Nagelamide R (**17**) also showed antimicrobial activity against *B. subtilis, Trichophyton mentagrophytes, C. albicans,* and *A. niger* with MIC values of 13.0, 6.0, 13.0 and 13.0 μg/mL, respectively.[10]

Table 1. Antibacterial and antifungal activity of Nagelamides

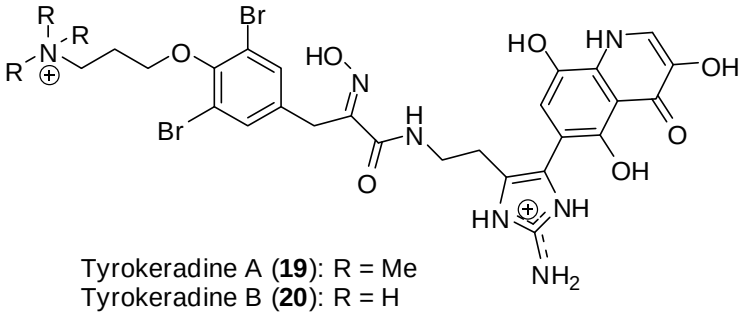
	Antibacterial and antifungal activity (MIC, μg/mL)						
Comp. No.	*M. luteus*	*B. subtilis*	*E. coli*	*S. aureus*	*C. neoformans*	*T. mentagrophytes*	*A. niger*
1	2.08	1.67	3.33	a	a	a	a
2	4.17	3.33	3.33	a	a	a	a
3	4.17	3.33	3.33	a	a	a	a
4	4.17	3.33	3.33	a	a	a	a
5	4.17	1.67	3.33	a	a	a	a
6	4.17	1.67	3.33	a	a	a	a
7	2.08	1.67	3.33	a	a	a	a
8	1.67	3.33	>3.33	a	a	a	a
9	a	a	a	8.35	16.7	a	a
10	16.7	a	a	a	a	a	a
11	16.7	a	a	a	a	a	a
12	a	a	a	a	a	a	33.3
13	a	a	a	a	a	a	33.3
14	33.3	33.3	a	33.3	a	a	
16	13.0	a	a	a	13.0	6.0	13.0
17	a	a	a	a	13.0	6.0	13.0

a = not tested

Agelamide[11] (**18**), a fluorescent alkaloid, was isolated from hydrophilic extract of marine sponge *Agelas nakamurai* as matrix-metalloproteinase 2 inhibitor. Agelamide inhibited 33.3% cell migration *in vitro* using bovine aortic endothelial cells at 5 μg/mL and 65.9% at 25 μg/mL, respectively. 10 μg/mL concentration of **18** inhibited vascular formation from aggregates of vascular progenitor cells in 3D culture using type-1 collagen gel.

4.1.2.2 Tyrokeradines:

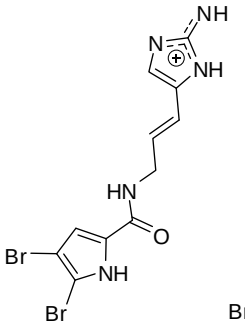
Mukai et al. isolated two rare bromotyrosine alkaloids, Tyrokeradine A (**19)** and B (**20)** possessing an imidazolyl-quinolinone moiety, from Okinawan Verongid sponge (SS-301).[12] Tyrokeradine B (**20**) inhibited the growth of *M. luteus, Staphylococcus aureus, T. mentagrophytes* with same MIC value of 25.0 μg/mL and was more active against *C. neoformans, C. albicans, and A. niger* with MIC values 12.5 μg/mL each. While **19** was less active than **20** as antimicrobial agent, indicating that free amino group at end of aliphatic tail is not favourable for antimicrobial activity.



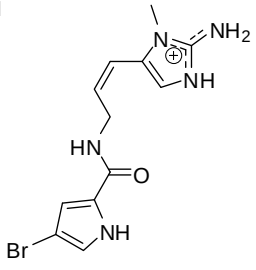
Tyrokeradine A (**19**): R = Me
Tyrokeradine B (**20**): R = H

4.1.2.3 Oroidins:

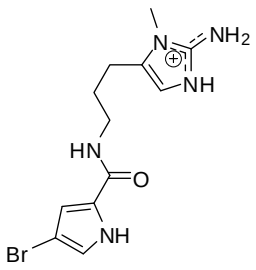
Oroidin (**21)** was first isolated[13,14] from *Agelas oroides* and then from various sponges.[15,16,17,18,19,20,21,22,23,24] It exhibited good antifouling activity and inhibited the larval metamorphosis of barnacle *Balanus amphitrite* with ED_{50} value of 19 μg/mL but was moderate in antibacterial activity with growth inhibitory zone of 15 mm at 10 μg/disk.



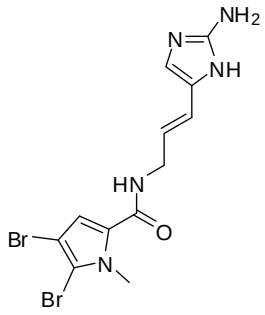
Oroidin (**21**)



Keramidine (**22**)



Dihydrokeramidine (**23**)

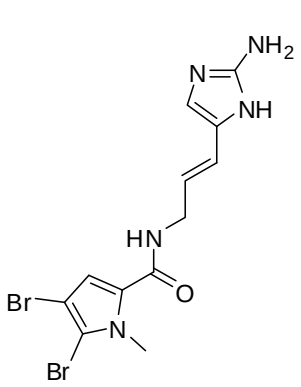


Sventrin (**24**)

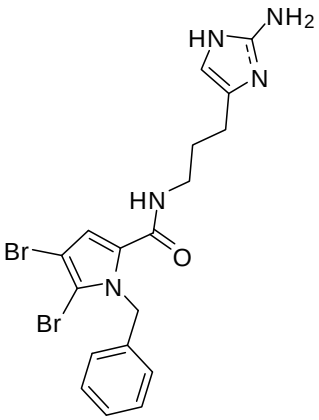
Z-isomer of oroidin with methyl at one nitrogen of guanidine part i.e. Keramidine (**22**), and 9,10-dihydrokeramidine (**23**)[25] were screened against *M. luteus*, *B. subtilis*, *E. coli*, protein phosphatise 2 A, but were found to be inactive.[5] Oroidin (**21**) was

more potent PfFab1 inhibitor[26] (IC_{50} = 0.30 μg/mL) than E-oroidin's TFA salt. Oroidin also inhibited the larval metamorphosis of the barnacle *Balanus amphitrite* at ED_{50} value of 19 μg/mL. It also showed inhibitory zones of 15 mm against *Flavobacterium marinotypicum*[22] at 10 μg/disk and non toxic at a concentration of 30 μg/mL. It is a non competitive, non specific antagonist of histamine receptor with pD2 value of 4.02 ± 0.11.[21]

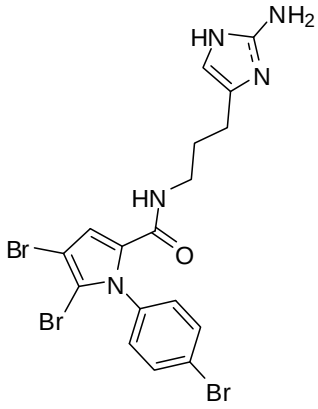
Bioassay guided fractionation of sponge *Agelas sventres* collected off the coast of North Cat Cay, Bimini, Bahamas by Köck et al. led them to isolation of a new bromopyrrole alkaloid Sventrin (**24**).[27] Like oroidin, sventrin also deterred feeding of the Caribbean reef fish *Thalassoma bifasciatum* in aquarium assays, but was less active than oroidin.



Dihydrosventrin (**25**)



(**26**)

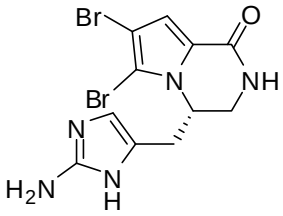


(**27**)

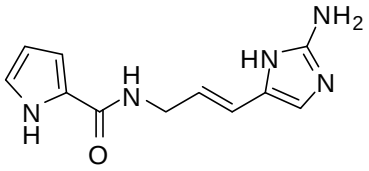
Melander et al. in their efforts toward identification of simple oroidin analogues[28] having potent antibiofilm activity found dihydrostventrin (DHS) (**25**) as a very potent inhibitor of *Pseudomonas* biofilm formation with no direct antibiotic effect with IC_{50} values of (51 ± 9) and (111 ± 8) μM against PAO1 and PA14, respectively. All other variations in chain length, introduction of unsaturation in linker, removal of bromopyrrole part had detrimental effect on antibiofilm activity. Replacement of 2-aminoimidazole with similar five membered ring system also resulted in substantial decrease in activity. For SAR of the lead compound oroidin, Melander and co-workers studied the effect of reversal[29] of amide bond which connects the bromopyrrole part with 2-aminoimidazole ring. They found that in reverse amides,

replacing bromopyrrole part with long chain aliphatic chain, increases activity substantially.

Excited by the discovery of DHS as the first non toxic small molecule inhibitor of biofilm formation in a mucoid variant of *Pseudomonas aeruginosa*, Melander's group synthesized and evaluated a few DHS derivatives.[30, 31] They found that replacing *N*-methyl in DHS with benzyl or 4-bromophenyl increases the potency of biofilm inhibition. These analogues **26** & **27** were more active as biofilm inhibitors than as biofilm dispersal agent.



Cyclooroidin (**28**)

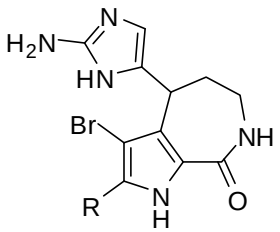


(**29**)

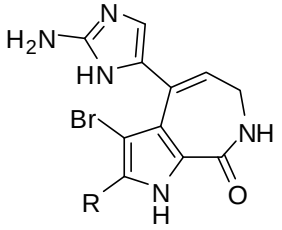
Cyclooroidin (**28**) isolated from Mediterranean sponge *Agelas oroides*, was tested on isolated guinea pig ileum for anticholinergic, antiserotonergic, antihistaminic activity but showed no activity.[32] Cytotoxic alkaloid **29** was isolated from *Agelas clathrodes* collected near Desecheo Island, Puerto Rico, in March 1989.[33]

4.1.2.4 Hymenin:

Antihistaminic bromopyrrole alkaloid hymenin (**30**) was first isolated in 1986.[17,34] Proschk and co-workers isolated debromohymenin (**31**) from sponge *Stylissa carteri*



Hymenin (**30**): R = Br
Debromohymenin (**31**): R = H



Stevensine (**32**): R = Br
2-Debromostevensine (**33**): R = H

collected in 1997 at Ambon and Sulawesi, Indonesia.[35] Hymenin is a competitive antagonist of α-adrenoceptors in vascular smooth muscles of isolated aorta of rabbit. Alkaloid **31** was proved to be inactive against human monocytic leukemia cells

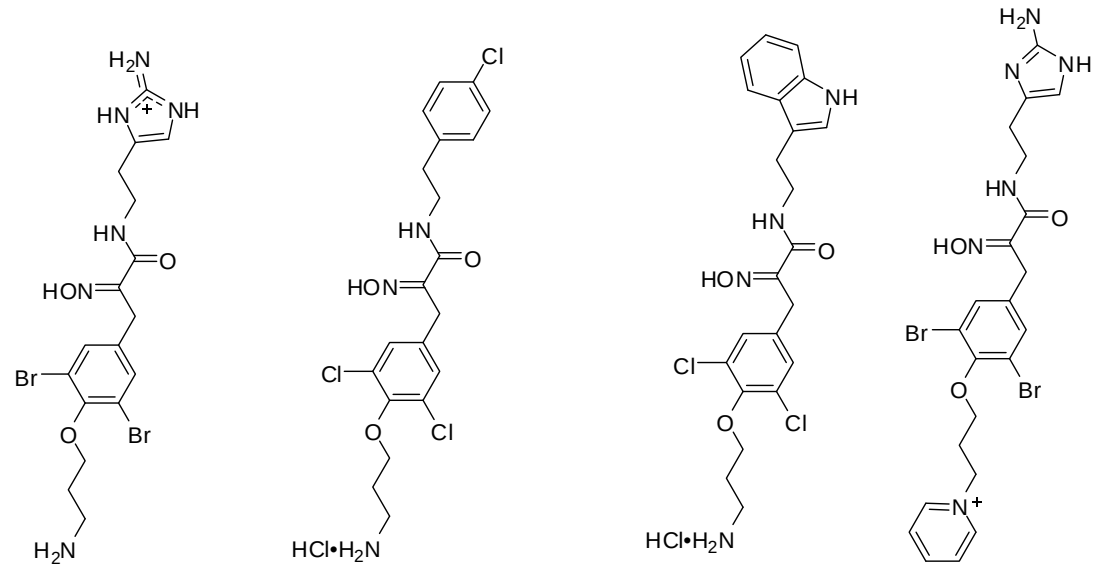
(MONO-MAC 6) in cytotoxicity assay. Compound **30** did not inhibit the mitogen-activated protein kinase-1.[36]

4.1.2.5 Stevensin:

Stevensin (**32**) was isolated by Faulkner's group in 1985 from methanolic extract of an unidentified Micronesian marine sponge.[37,38] Proschk and co-workers isolated debromostevensin (**33**) as an orange amorphous solid from methnaolic extract of marine sponge *Styllisa carteri (syn Axinella carteri)* collected in 1997 at Ambon and Sulawesi, Indonesia.[35] Both stevensin (**32**) and debromostevensin (**33**) were inactive against human monocytic leukemia cells (MONO-MAC 6).

4.1.2.6 Purealidins:

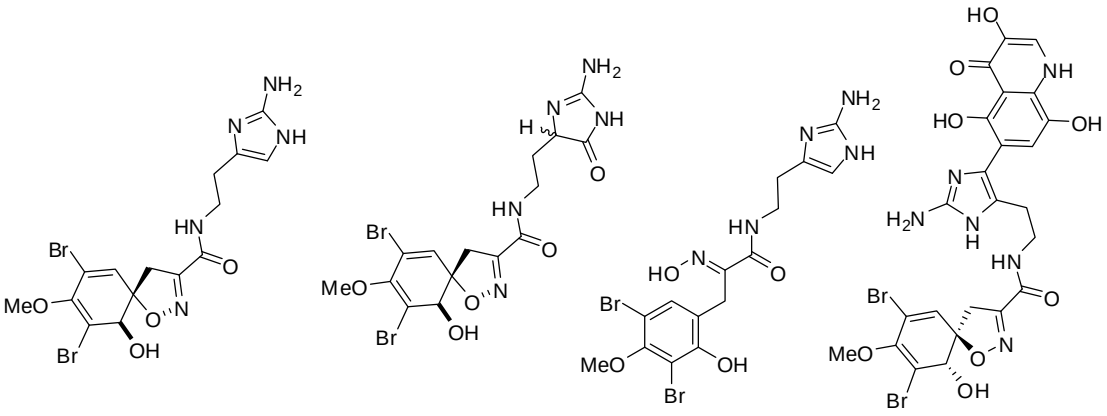
Purealidin A (**34**) was isolated from Okinawan Sponge *Psammaplysilla purea.*[39] It inhibits MSH dependant detoxification of enzyme mycothiol-S-conjugate amidase with IC_{50} value of (32 ± 3) μM.[40] Day and co-workers synthesized a small library of



Purealidin A (**34**) (**35**) (**36**) Purealidin D (**37**)

purealidin A analogues and found that purealidin A (**34**) itself was inactive against human cancer cell lines as antiproliferative agent but its analogues **35** & **36** exhibited antiproliferative activity in low micromolar range and alkaloid **34** also inhibited the MT-stimulated ATPase activity of a recombinant form of the full motor domain fragment of the rat cytoplasmic dynein heavy chain.[41] Low cytotoxicity and selective

inhibition of cytoplasmic dynein ATPase by alkaloid **34** proved that it is a good lead for discovery of small molecule inhibitors of cytoplasmic dynein heavy chain.



Purealidin J (**38**) Purealidin K (**39**) Purealidin M (**40**) (**41**)

Purealidin D (**37**), first alkaloid with pyridinium and bromotyrosine moiety of marine origin, was isolated from sponge *Psammaplysilla Purea.*[42] Biological activity of **37** is not reported yet.

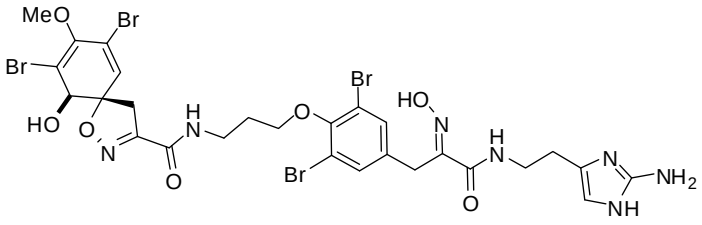
Purealidins J (**38**), K (**39**), and M (**40**), the bromotyrosine alkaloids were isolated from the Okinawan marine sponge *Psammaplysilla purea* collected of Ishigaki Islands, Okinawa.[43] Purealidin J and K moderately inhibited the activity of epidermal growth factor receptor (EGFR) kinase with the IC_{50} values of 23 and 14 μg/mL, respectively. Purealidin J was non toxic to ovarian tumour and leukemia cell lines.[44,45]

Alkaloid **41** is a rare example of an 2-aminoimidazole coupled to another aromatic substituent, was isolated by bioassay guided purification of MeOH/10%H_2O soluble sample from *Oceanapia* sp.[46] It was found to be a good inhibitor of mycothiol S-conjugate amidase, which would permit blocking of mycothiol dependant detoxification. So, this alkaloid can act as important lead for drug development against tuberculosis.

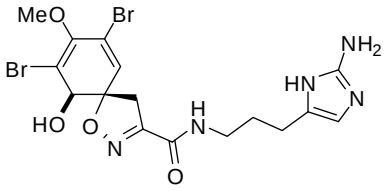
4.1.2.7 Purealin:

Pureallin (**42**) was also isolated with the purealidin from the same source. It inhibited the ATPase activity of isolated axonemal dynein and skeletal muscle myosin without competing for the ATP sites on these motor proteins.[47,48,49] A few purealin analogues were also synthesized by Day et al. and were found to have good antiproliferative

activity against mouse leukemia cell lines, but showed selective and moderate activities against human carcinoma.[41]



Purealin **(42)**



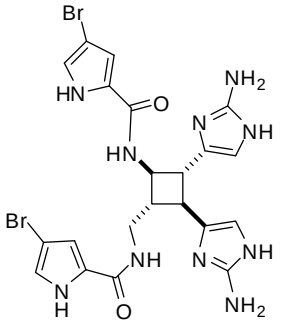
Aerophobin-2 **(43)**

4.1.2.8 Aerophobin-2:

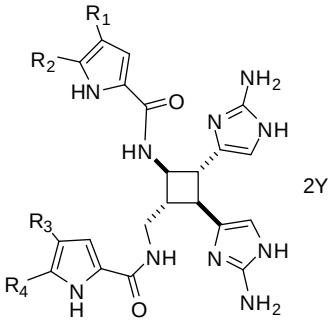
Aerophobin-2 (**43**) was initially isolated from marine sponge *Verongia aerophoba*.[50] Later it was isolated from *Aplysina aerophoba* and *Aplysina cavericola*.[51] A few years later Jaspar's group also isolated it from *Druinella sp.* and screened against ovarian tumor and leukemia cells but was found to be inactive.[52]

4.1.2.9 Sceptrin:

Sceptrin (**44**) was first isolated by Faulkner et al. as water soluble alkaloid from marine sponge *Agelas sceptrum*.[53] It is an antimicrobial agent active against *S. aureus, B. subtilis, C. albicans* with MIC value of 10 μg/mL each. Reinhart's group

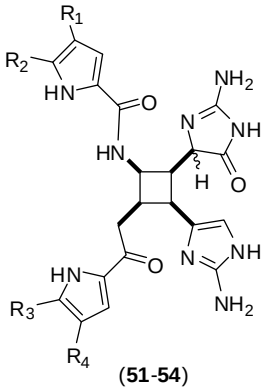


Sceptrin **(44)**



(**45**-**50**)

	R_1	R_2	R_3	R_4	Y
(**45**)	H	H	H	H	HCl
(**46**)	Br	H	H	H	HCl
(**47**)	Br	H	H	H	AcOH
(**48**)	Br	H	H	Br	AcOH
(**49**)	Br	H	H	Br	HCl
(**50**)	Br	Br	Br	Br	AcOH



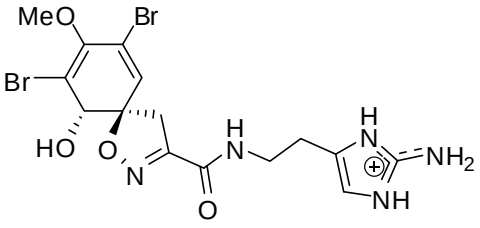
(**51**-**54**)

	R_1	R_2	R_3	R_4	Y
(**51**)	Br	Br	Br	Br	AcOH
(**52**)	H	H	H	Br	AcOH
(**53**)	Br	H	H	Br	AcOH
(**54**)	Br	H	H	Br	HCl

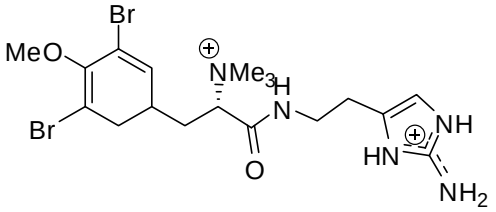
reported that sceptrin isolated from *Agelas conifera* has weak to moderate antiviral and antibacterial activity.[24] Sceptrin also showed non-competitive antagonism towards histamine receptors with pD2 value of (4.24 ± 0.09).[51] It showed

antibacterial activity against *M. luteus*, *B. subtilis*, *E. coli* with MIC values of 4.07, 8.33 and 33.3 μg/mL, respectively. It also inhibited protein phosphatise type 2A with IC_{50} value of 50 μM. Sceptrin was moderately active against *C. neoformans*[54] with an IC_{50} value of 3.5 μg/mL. Debromosceptrin (**45**), a symmetrical pyrrole dimer, was isolated from ethanolic extract of *Agelas conifer*, together with other analogues (**45-50**). Compounds (**45-50**) were inactive as anti-HIV, antimalarial and cytotoxic agent. But compounds **47** & **49** showed marginal activity against *Mycobacterium tuberculosis* with MIC values of 12.5 μg/mL each.[55]

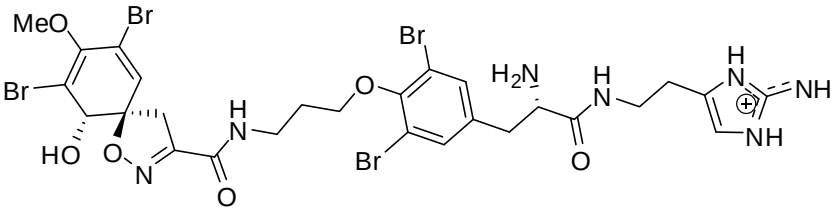
Oxysceptrin (**51**) was isolated from Okinawan marine sponge *Agelas nemoechinata* and *Agelas conifera* as potent actinomyosin ATPase activator activity.[56,57] Compounds (**51-54**) inhibited growth of *B. subtilis* at 10 μg/disk with (**51-53**) being somewhat less active. Compound **54** was also active against *E. Coli* at 10 μg/disk. All the sceptrin analogues were active against Herpes Simplex virus type 1 at 20 μg/disk and against vesicular stomatitis virus at 10 μg/disk while (**51-54**) were less active against both viruses. All the sceptrin and oxysceptrin analogues were non toxic to monkey kidney cells at 200 μg/disk.[43]



Pseudoceratinine A (**55**)



Pseudoceratinine B (**56**)



Pseudoceratinine C (**57**)

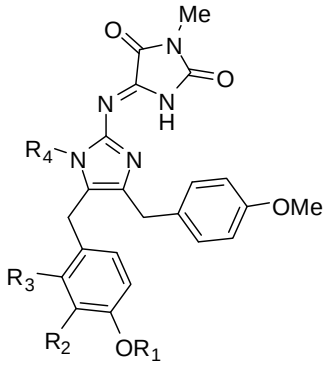
4.1.2.10 Pseudoceratinines:

Pseudoceratinine A (**55**) and B (**56**) were isolated from two specimens of *Psedoceratina verrucosa* collected at Ile Longue, but pseudoceratinine C (**57**) was isolated from the specimen collected of Ile Walpile.[44] Only pseudoceratinin A

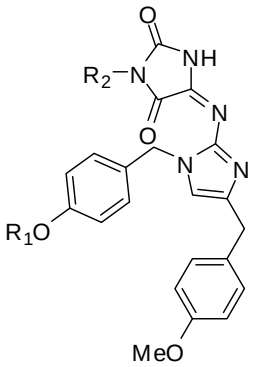
inhibited MSH dependent detoxification enzyme mycothiol-S-conjugate amidase with IC_{50} value of (100 ± 21) μM.[40]

4.1.2.11 Naamidine:

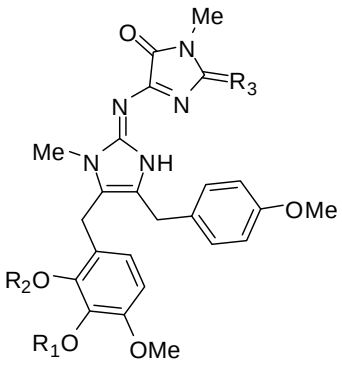
Naamidine A (**58**), Isonaamidine B (**63**),[58] Isonaamidine C (**64**)[59] were isolated from EGF dependent DNA synthesis and cell proliferation inhibitory ethanolic extract of a bright yellow sponge *Leucetta* sp.[60] Among these three, only naamidine A (**58**) was a



Naamidine A (**58**): R_1, R_2, R_3 = H, R_4 = H
Naamidine B (**59**): R_1 = Me, R_2 = OH, R_3 = H, R_4 = Me
Naamidine D (**60**): R_1, R_2, R_3 = H, R_4 = Me
Naamidine G (**61**): R_1 = Me, R_2, R_3 = H, R_4 = Me
Pyronaamidine (**62**): R_1 = CH_3,R_2 = OCH_3, R_3 = OH, R_4 = Me



Isonaamidine B (**63**): R_1 = H, R_2 = Me
Isonaamidine C (**64**): R_1 = Me, R_2 = Me
Isonaamidine D (**65**): R_1 = H, R_2 = H



Naamidine H **(66):** R_1 = H, R_2 = Me, R_3 = O
Naamidine I **(67):** R_1 = H, R_2 = Me, R_3 = NMe
(2E, 9E) Pyrronaamidine-9-(n-methylimine) (**68**): R_1 = Me, R_2 = H, R_3 = NMe

potent inhibitor of epidermal growth factor (EGF)-stimulated DNA synthesis[61] and it act by targeting extracellular signal regulated kinase ERK 1 and ERK 2.[62] On the basis of these finding, Watson's group synthesized naamidine A and confirmed EGF

inhibitory activity. A series of its derivatives were also synthesized and evaluated for their ability to inhibit mitogenesis in BaF/ERX cells. It was also discovered that 4-methoxybenzyl substituent of natural product was unnecessary for activity and replacement of 2-aminoimidazole part with thiazole also doesn't affect the biological activity but none of the synthesized analogues was more active than naamidine A (**58**).[63]

Naamidine A (**58**), B (**59**), and G (**61**) are antifungal agents with activity against *Cryptococcus neoformans* with MIC values of 12.5, 6.25 and 12.5 μg/mL, respectively, while naamidine D (**60**) was not tested.[64]

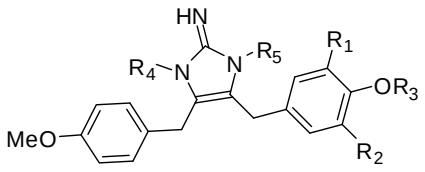
Naamidine H (**66**) and I (**67**) were isolated from the marine sponge *Leucetta chagosensis* collected in North Sulawesi, Indonesia. Naamidine H and I were cytotoxic against HeLa cells with IC_{50} values of 5.6 and 15 μg/mL, respectively.[65] (2E, 9E) Pyrronaamidine-9-(n-methylimine) (**68**) was isolated from yellow sponge *Leucetta* cf *chagosensis* collected near the Island of Rota, Northern Mariana Islands. It is mildly toxic toward A-549, MCF-7, HT-29 cell lines with GI_{50} values of 6, 3 and 6 μg/mL, respectively.[66] Pyrronaamidine (**62**) has weak antimicrobial activity against *B. subtilis* and *C. albicans* with 10 and 7 mm zones of inhibition at a concentration of 100 μg/disk.[66]

4.1.2.12 Naamines:

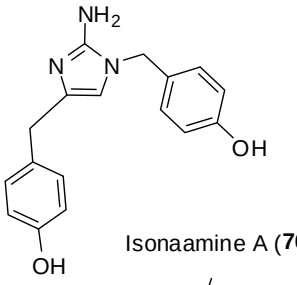
Naamine A (**69**),[58] isonaamine A (**76**) along with naamidine A (**58**), isonaamine A (**76**) were isolated from methanolic chloroform extract of the sponge *Leucetta chagosensis*.[59] Naamine C (**71**) was isolated from same sponge but by different group. New cytotoxic 2-aminoimidazole analogues (**78**), isonaamine C (**79**), (**80**) were isolated from *L. chagosensis* collected from the Great Barrier Reef and Fiji islands.[67] Upon cytotoxicity evaluation alkaloid **78** and isonaamine C were found to be cytotoxic with GI_{50} values of 1.3 and 2.1 μg/mL against Huh7 cell line, respectively.

Isonaamine B (**77**) was also isolated from the same source and was inactive as antimicrobial agent.[68] Chemical investigation of extract of sponge *Leucetta chagosensis* collected in Chuuk State afforded a 2-aminoimidazole alkaloid named as naamine C (**71**), biological activity of which is yet to be reported.[69] Naamine D (**72**)

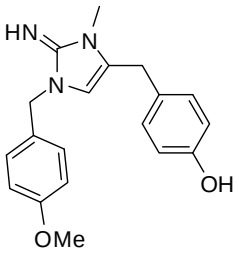
was isolated from Egyptian Red Sea Sponge *Leucetta* cf *chagosensis* and it has moderate antifungal activity against AIDS-O1 pathogen *C. neoformans* with MIC value of 6.25 μg/mL and nitric oxide synthetase inhibitory activity with 50% reduction in production at 1.0 mM.[64]



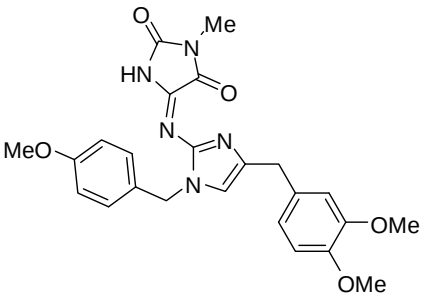
Naamine A (**69**): R_1 = OH, R_2, R_3, R_4 = H, R_5 = Me
Naamine B (**70**): R_1 = OH, R_2, R_4, R_5 = H, R_3 = Me
Naamine C (**71**): R_1 = OH, R_2, R_4 = H, R_3, R_5 = Me
Naamine D (**72**): R_1,R_2, R_4, R_5 = H, R_3, = Me
Naamine E (**73**): R_1,R_2= OH, R_3, R_5 = Me, R_4 = H
Naamine F (**74**): R_1 = OMe, R_2, R_3, R_4 = H, R_5 = Me
Naamine G (**75**): R_1,R_2= OMe, R_3, R_4 = H, R_5 = Me



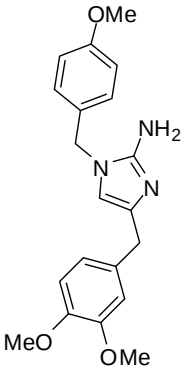
Isonaamine A (**76**)



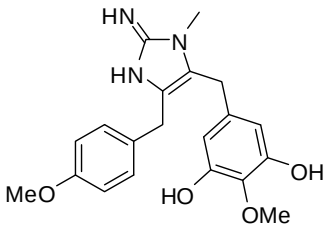
Isonaamine B (**77**)



(78)



Isonaamine C (**79**)



(**80**)

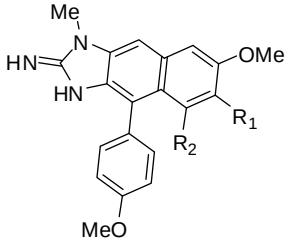
Naamine F (**74**) and G (**75**)[70] were isolated from *L. Chagosensis* collected at South Sulawesi, Indonesia. Naamine G (**75**) is a good antifungal agent with MIC value of 20 μg/disk showing inhibition of 20 mm zone in agar plate diffusion assay of *C. herbarum*. Naamine G (**75**) also exhibited moderate cytotoxicity toward Lymphoma (L5178Y) and human cervix carcinoma (HeLa) with 46 and 29% inhibition, respectively but was inactive toward rat brain tumor.

4.1.2.13 Kealiinines:

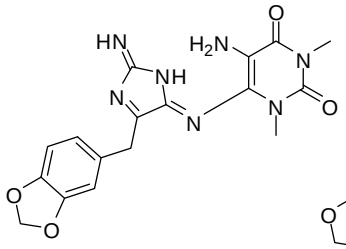
Kealiinines (**81–83**) were isolated from the same source as that of naamine F and G. Only kealiinine A (**81**) showed 50 % mortality rate in the brine shrimp lethality test.[70]

4.1.2.14 Leucosolenamine:

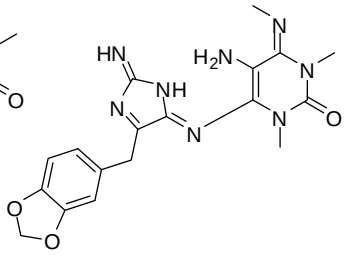
Leucosolenamine A (**84**) and B (**85**) were isolated from Leucosolenia genus collected at Papua New Guinea. Leukosolenamine A was mildly cytotoxic with 10.5 mm zone of inhibition of murine C-38 cells at 180 μg/disk as compared to 0 mm against CFU-GM cells indicacting it as mildly potent selective cytotoxin.[71] But alkaloid **85** was inactive.



Kealiinine A (**81**): R_1 = OMe, R_2 = H
Kealiinine B (**82**): R_1 = OH, R_2 = H
Kealiinine C (**83**): R_1 = OMe, R_2 = OMe



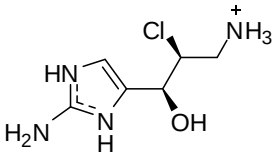
Leucosolenamine A (**84**)



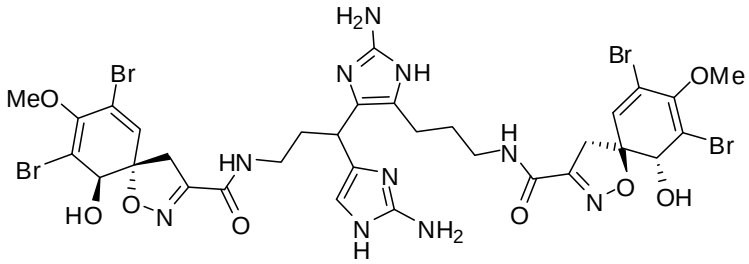
Leucosolenamine B (**85**)

4.1.2.15 Girroline:

Girroline (**86**) an antitumor alkaloid, was isolated from New Caledonian sponge *Cymbastella cantharella* (previously *Pseudaxinyssa cantharella*)[72],[73] which showed both *in vitro* and *in vivo* antitumour activity. [74] It arrests the cell cycle in G2/M phase in several tumor cell lines and accumulates polyubiquitinated p53 at least in FL cells.[75] Because of several unfavourable side effects, clinical trials of compound **86** in phase 1 were stopped. Girroline (**86**) also exhibited good antimalarial activity with



Girroline (**86**)



Archerine (**87**)

IC_{50} values in range of 77 to 215 nM against 4 strains of *Plasmodium falciparum*.[76] It inhibited growth of parasite by 100% with artemisinin and chloroquine. Girroline was active at a dose of 1mg/kg /day *in vivo* by both oral and i.p. route and targeted the

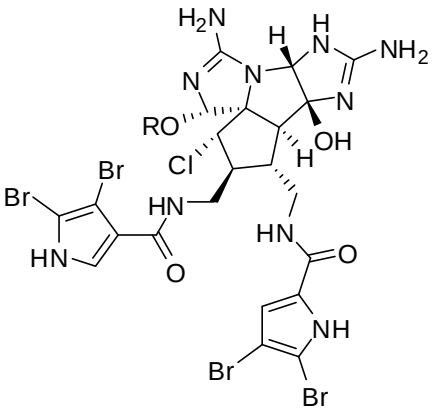
synthesis of proteins by the parasite. Therefore, girroline can be considered as potential lead structure for antiplasmodial drug research.

4.1.2.16 Archerine:

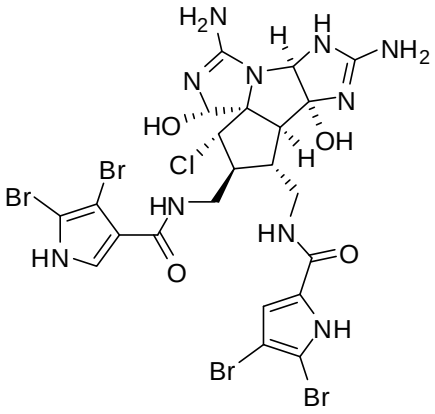
Archerine (**87**) was isolated from a typical West Indian species *Aplysina archeri* which grows in reef habitat at a depth of 2-40 m.[77] It showed reversible antagonism of histamine when assessed using histamine induced contraction of guinea pig isolated ileum at 1.2×10^{-4} M. Archerine doesn't have all the structural features which are currently used as guidelines for synthesis of antihistaminic drug.

4.1.2.17 Axinellamines:

Axinellamines A–D (**88–91**) were isolated by bioassay guided fractionation of crude methanol extract of Australian marine sponge *Axinella* sp.[78] Axinellamine B (**89**), C (**90**), D (**91**) showed antibacterial activity against *Helicobacter pylori*, a gram negative bacteria associated with peptic and gastric cancer, with MIC value of 1000 μM. Axinellamine A (**88**) has no bactericidal activity at 1000 μM.



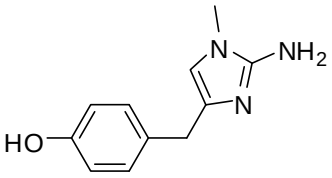
Axinellamine A (**88**): R = H
Axinellamine B (**89**): R = Me



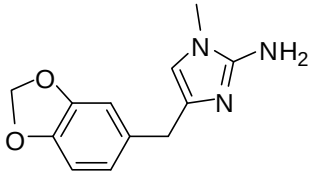
Axinellamine C (**90**): R = H
Axinellamine D (**91**): R = Me

4.1.2.18 Dorimidazole A:

Dorimidazole A (**92**), the simplest example of 2-aminoimidazole alkaloid having antiparasitic activity against *Nipporstrongylus brasiliensis* at 50μg/mL was isolated from Indopacific nudibranch but it's synthetic sample (HBr salt) was inactive.[79]



Dorimidazole A (**92**)



Preclathridine A (**93**)

4.1.2.19 Preclathridine:

Chemical investigation of nudibranch, *N. guardineri*, collected from Papua New Guinea resulted in isolation of the secondary metabolite preclathridine A (**93**).[80] Biological activity of preclathridine A is not yet reported.

4.1.2.20 Ageliferins:

Ageliferin (**94**), bromoageliferin (**95**), dibromoageliferin (**96**) were isolated from Okinawan marine sponge *Agelas* sp. and found to be potent actomyosin ATPase activator.[24,81] ATPase activity of myofibrils from rabbit skeletal muscles was elevated to 150, 190 and 200% of the control value by ageliferin (3×10^{-5}), bromoageliferin (10^{-6}), and dibromoageliferin (10^{-6}). But later on (**94–96**) were also isolated from

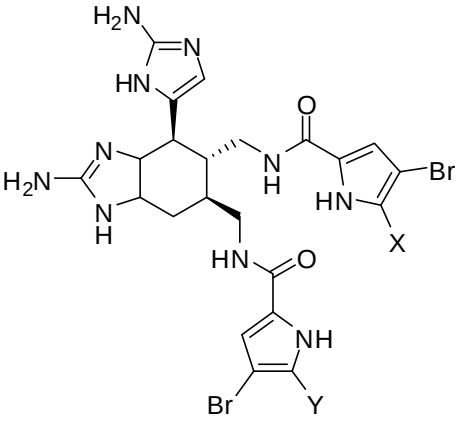
Table 2. Antimicrobial and phosphatase type 2 A inhibitory activity[5] of ageliferins

Compound No.	Antibacterial activity (MIC, µg/mL)		
	M. luteus	*B. subtilis*	*E. coli*
Ageliferin (**94**)	4.17	8.33	33.3
Bromoageliferin (**95**)	2.08	2.08	16.7
Dibromoageliferin (**96**)	2.08	4.16	16.7

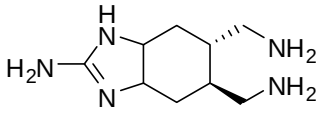
Agelas conifera and screened as antiviral, antibacterial agents and activity in barnacle settlement and biochemical prophage induction assay. Ageliferin was also found to inhibit important neurotransmitters such as vasoactive intestinal peptide (VIP) and somatostatin (somatotropin release inhibiting factor, SRIF) with IC_{50} values of 2.2 and 19.2 µM, respectively.[82]

Taking structural insights from bromoageliferin (**95**), Melander and his group hypothesized that bicycle core of bromoageliferin is key pharmacophore that imparts biological activity,[83] they designed two analogues of bromoageliferins TAGE (**97**)

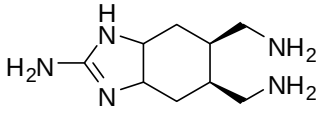
and CAGE (**98**). Both inhibited biofilm formation of *Pseudomonas aeruginosa* with IC_{50} values of 100 and 180 μM against PA O1 for TAGE and CAGE, respectively. While IC_{50} values were 100 and 190 μM against PA14 for TAGE and CAGE respectively. At 500 μM TAGE showed more than 50% reduction in planktonic growth while CAGE showed it at both 400 and 500 μM.



Ageliferin (**94**): X= Y = H
Bromoageliferin (**95**): X = Br, Y = H
Dibromoageliferin (**96**): X = Y = Br



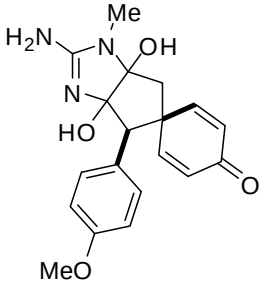
TAGE (**97**)



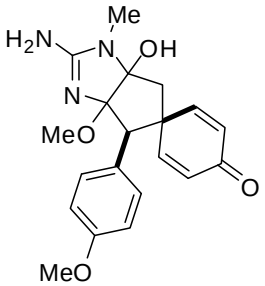
CAGE (**98**)

4.1.2.21 Calcaridine:

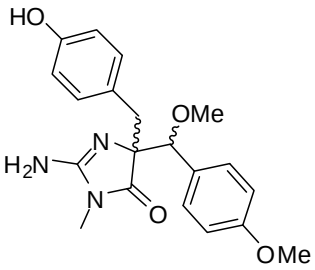
Calcaridines represents the first nonorganometallic chiral 2-aminoimidazole alkaloids isolated from calcareous sponges. (+) Calcaridine A (**99**), (-) spirocalcaridine A (**100**), (-) spirocalcaridine B (**101**) were isolated from Calcareous sponge *Leucetta* sp. collected at several sites south of Vitu, Levu, Fiji. (+) Calcaridine A posses geminally fused 2-aminoimidazolidinone, while (-) spirocalcaridine A is unique in having hexahydrocyclopentamidazol-2-ylidenamine spiro-linked to a cyclohexenone.[84,85] These alkaloid have not screened against any of the disease targets.



(-) Spirocalcaridine A (**99**)



(-) Spirocalcaridine B (**100**)

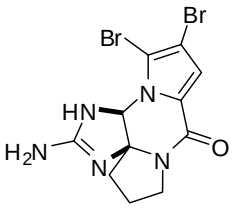


(+) Calcaridine A (**101**)

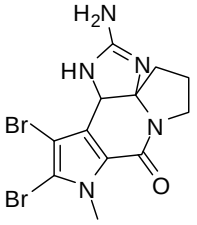
4.1.2.23 Phakellines:

Phakelline (**102**), bromophakelline (**103**), dibromophakelline (**104**) were isolated from marine sponge *Phakellia flabellata***.**[86,87] *N*-methyldibromoisophakelline (**105**) was isolated from methanolic extract of *Styllisa caribica* collected off the coast of Sweeting Cay, Bahamas as chemical defense metabolite. It is the only component in sponge tissue of *Styllisa caribica* which is responsible for chemical defense.[88]

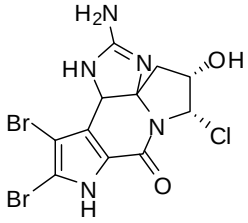
Antifeeding activity of few aminoimidazole alkaloids is in order sceptrin > N-methyldiboromoisophakellin > oroidin expressed as mole/mL. 12-Chloro-11-hydroxydibromoisophakelline (**106**) was isolated from marine sponge *Axinella brevistyla* collected in Western Japan. It exhibited antifungal activity against erg6 mutant type of the yeast *Sachromyces cerevisae* at 30 µg/disk. It was also cytotoxic against L1210 cells with IC_{50} value of 2.5 µg/mL.[89]



Phakellin (**102**): R_1, R_2 = H
Bromophakellin (**103**): R_1 = H, R_2 = Br
Dibromophakelline (**104**): R_1, R_2 = Br



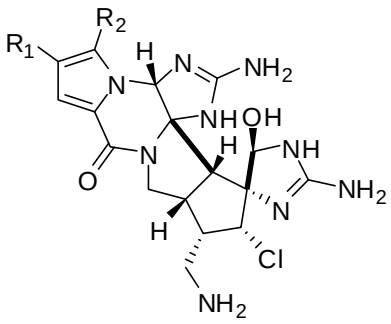
N-methyldibromo isophakelline (**105**)



12-chloro-11-hydroxydibromo isophakelline (**106**)

4.1.2.24 Palau'mines:

Palaúmine (**107**) was isolated from sponge *Stylotella aurantium* collected in Western Caroline Islands. Palaúmine exhibited antitumor activity against P388 and A549 cells with IC_{50} values of 0.1 and 0.2 µg/mL, respectively. It also showed antibiotic activity against *Penicillium notatum* with 24 mm zone of inhibition at 50 µg/disk. Palaúmine

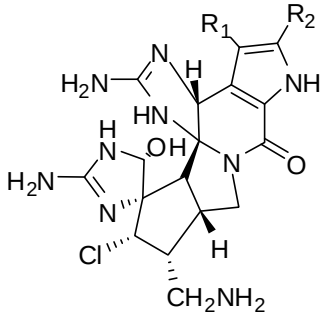


Palaúamine (**107**): R_1, R_2 = H
Bromopalaúmine (**108**): R_1 = H, R_2 = Br.
Dibromopalaúmine (**109**): R_1, R_2 = Br

showed immunosuppressive activity also with IC_{50} < 18 ng/mL in the mixed lymphocyte reaction.[20] 4-Bromopalaúmine (**108**) and 4,5-dibromopalaúmine (**109**) were isolated a few years later from the same sponge by the same group.[57] Both of these derivative were less active in comparison to palaúmine itself, but 4,5-dibromo derivative was selective against a human melanoma cell line with an IC_{50} value of 0.25 μg/mL.

4.1.2.25 Styloguanidines:

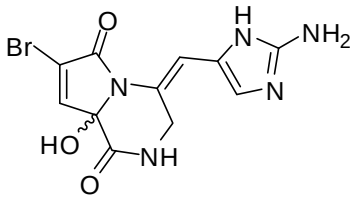
Styloguanidines (**110–112**), regioisomers of palaúmine, were isolated from marine sponge *Stylotella aurantium* collected off Yap sea. All styloguanidines, also known as isopalaúmines, inhibit chitinase, most important enzyme for ecdysis of crustaceans through hydrolysis of integumental chitin, of *Schwanella* sp. at a concentration of 2.5 μg/disk. These compounds also showed antifouling activities against barnacles by inhibiting the moulting of their cyprid larvae at 10 ppm.[90]



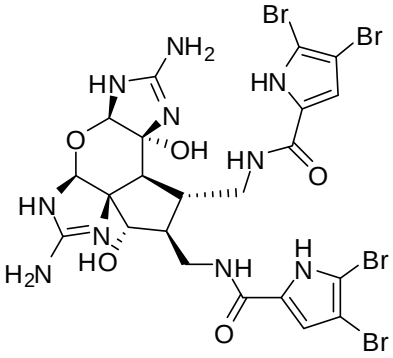
Styloguanidine (**110**): R_1, R_2 = H
Bromostyloguanidine (**111**): R_1= Br, R_2 = H
Dibromostyloguanidine (**112**): R_1, R_2 = Br

4.1.2.26 Oxycyclostylidol:

Oxycyclostylidol (**113**) was isolated from Caribbean sponge *Stylissa caribica*, is the first example of twice oxidised pyrrole-imidazole alkaloid from natural source. Oxycyclostylidol showed minor activity against several pathogenic bacteria, virus, fungi and culture of mice fibroblasts.[91]



Oxycyclostylidol (**113**)



Massadine (**114**)

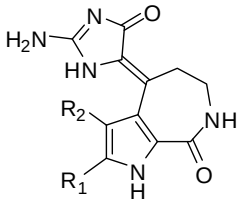
4.1.2.27 Massadine:

Massadine (**114**) was discovered by bioassay guided purification of organic extract of marine sponge *Styllisa* aff. *massa* collected in Gulf of Sagami, as inhibitor of candida GGTase 1. It inhibited candida GGTase 1 with IC_{50} value of 3.9 μM. Massadine also inhibited the growth of *C. neoformans* with MIC value of 32 μM but was inactive against *C. albicans* even at a concentration of 64 μM.[24,92]

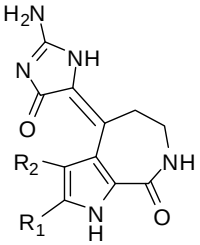
4.1.2.28 Hymenialdisines:

Hymenialdisine (**116**) was initially isolated from two marine sponges *Axinella verrucosa* and *Acanthella aurantiaca*.[16] A few other hymenialdisines were also isolated from *Stylissa massa* and *Styllisa carteri* by bioassay guided fractionation. Z-Debromohymenialdisine (**115**) and Z-hymenialdisine (**116**) were active against MONOMAC-6 (human monocytic leukemia cells) in cytotoxicity assay with IC_{50} values of 2.4 and 0.2 μg/mL, respectively.[35] E-Bromohymenialdisine (**120**), E-hymenialdisine (**119**), E-debromohymenialdisine (**118**) were isolated from marine sponge *Stylotella aurantium* collected off Palaú.[93] Hymenialdisine and debromohymenialdisine (**115**) showed insecticidal activity against neonate larvae of polyphagous pest insect *Spodoptera littoralis* with LD_{50} values of 88 and 125 ppm respectively, when incorporated in the artificial diet of the larvae in chronic feeding assay. Z-Hymenialdisine (**116**) and E-hymenialdisine (**119**) both showed good inhibitory activity against mitogen activated protein kinase kinase-1 with IC_{50} values of 3 and 6 nM, respectively.[36] They also inhibited the growth of human tumor LoVo cells. 3-Bromo-Z-hymenialdisine (**117**) was isolated from tropical marine sponge *Axinella carteri*.[94] Cytotoxicity studies against mouse lymphoma cells (L5178y)

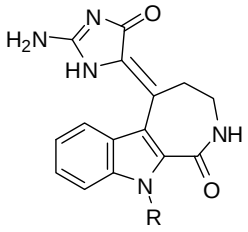
revealed that debromohymenialdisine (**115**) was most active with IC_{50} value of 1.8 μg/mL followed by hymenialdisine (**116**) and 3-bromohymenialdisine (**117**) which were equitoxic with IC_{50} values of 3.9 μg/mL. Hymenialdisine inhibited interleukin-8 production in U-937 cells by inhibition of nuclear factor-κB.[95] It also inhibited NF-κB mediated interleukin-1β-stimulated prostaglandin E2 formation.[96] Debromohymenialdisine (**115**) inhibited G2 DNA damage check point with an IC_{50} value of 8 μM and protein kinases chK1 and chK2 with 3 and 3.5 μM respectively.[97] So, debromohymenialdisine (**115**) may be used to sensitize the cancer cells toward DNA damaging therapies.



Z-Debromohymenialdisine (**115**):
$R_1 = R_2 = H$
Z-Hymenialdisine (**116**):
$R_1 = Br, R_2 = H$
Z-Bromohymenialdisine (**117**):
$R_1 = H, R_2 = Br$



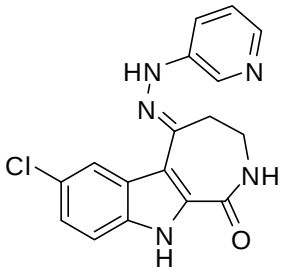
E-Debromohymenialdisine (**118**):
$R_1 = R_2 = H$
E-Hymenialdisine (**119**):
$R_1 = Br, R_2 = H$
E-Bromohymenialdisine (**120**):
$R_1 = H, R_2 = Br$



(121) R = H
(122) R = Me

Tepe's group synthesized indoloazepine derivatives **121** & **122** of hymenialdisine and screened them for cytokines IL-2 and TNF-α production.[98] They found hymenialdisine as good inhibitor of production of cytokines IL-2 and TNF-α with IC_{50} values of (2.41 ± 0.71) and (1.36 ± 0.25) μM, respectively but corresponding indoloazepine analogue **121** was less active with IC_{50} values of (3.55 ± 0.09) and (8.16 ± 0.31) μM against IL-2 and TNF-α production, respectively. *N*-methyl derivative **122** showed no significant inhibition of IL-2 or TNF-α production. Both of these indoloazepine analogues **121** & **122** were also inhibitors of Leukaemia T-cells growth with GI_{50} values of (1.61 ± 0.62), (1.73 ± 0.08) and (14.3 ± 2.41) μM for **121** and **122**, respectively. Both hymenialdisine (**116**) and **121** inhibited the binding of DNA to NF-κB of nuclear extract of PMA/PHA activated Jurkat cells. Hymenialdisine (**115**) was also discovered as a potent inhibitor of cyclin-dependant kinases, glycogen synthase kinase-3β and casein kinase-1 by Thurini and co-workers after screening various compounds isolated from marine sponges.[99] So,

hymenialdisine can act as a good lead compound in the treatment of neurodegenerative disorders.

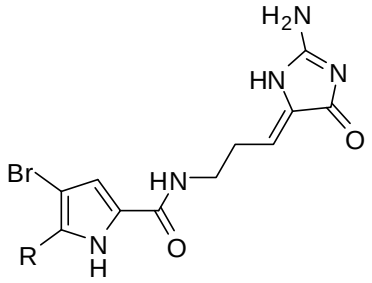


(**123**)

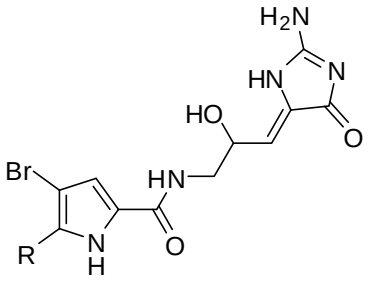
Gray's group designed and synthesized a few analogues of hymenialdisine taking insights from crystal structures of CDK-2 complexed with hymenialdisine.[100] They identified compound **123** as most potent which arrested the cells in G2/M phase at concentration as low as 3.8 μM and showed 30 fold higher antiproliferative activity than hymenialdisine (**116**).

4.1.2.29 Dispacamides:

Dispacamides, bromopyrrole alklaoids having 2-aminoimidazolone moiety, were isolated from four Caribbean *Agelas* sponges (*A. conifera, A. longissima, A. clathrodes, A. dispar*). Dispacamide A (**124**) and its debromoderivative dispacamide B (**125**) were inactive as anticholinergic as well as antiserotonergic agents but dispacamide A (**124**) was remarkably good and selective as antihistaminic agent when tested on the guinea pig ileum.[101] Structure activity relationship study of sponge derived and synthetic bromopyrrole alkaloids as inhibitors of fish feeding by Lindel et al. revealed that dispacamide A has antifeedant property against omnivorous Caribbean reef fish, *Thalassoma bifasciatum* but debromoderivative was inactive demonstrating importance of bromo group on pyrrole ring for antihistaminic as well as antifeedant activity.[102]



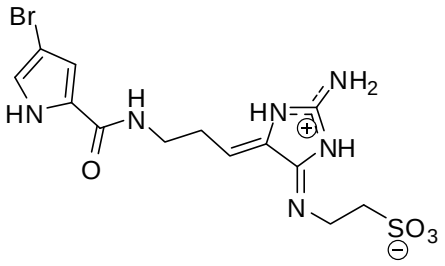
Dispacamide A (**124**): R = Br
Dispacamide B (**125**): R = H



Dispacamide C (**126**): R = Br
Dispacamide D (**127**): R = H

Dispacamide C (**126**) and D (**127**) showed a reversible, specific, non-competitive inhibition toward histamine receptors.[51] But dispacamides C and D were less active in comparison to that of A and B, with pD2 values of 4.48 ± 0.05 and 4.34 ± 0.10, respectively.

Chemical investigation of *Axinella verucosa* collected from Bay of Calvi (Corsica), yielded two dispacamide derivatives **128** and **129**.[103] Compound **128** was potent neuroprotective agents, good serotonin and glutamate antagonist, **129** also showed serotonin antagonism but was weaker than **128**. These two seems to be promising serotonin antagonists with a potential to treat psychosis, different phobia, and mood fluctuation disorders.



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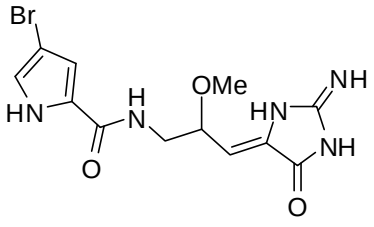
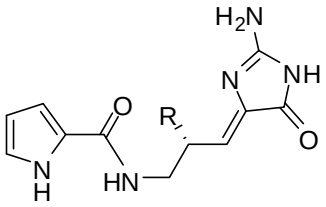
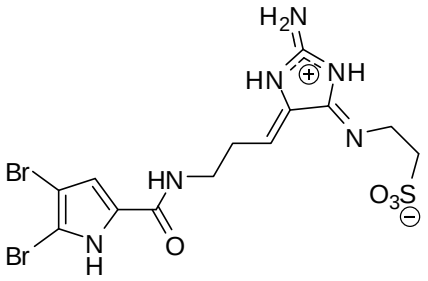


(129)

Debromodispacamides B (**130**) and D (**131**) were isolated from polar extracts of the sponge *Agelas mauritiana* collected off the Solomon Islands.[104] Biological activities of these two are yet to be reported.



Debromodispacamides B (**130**): R = H
Debromodispacamides D (**131**): R = OH

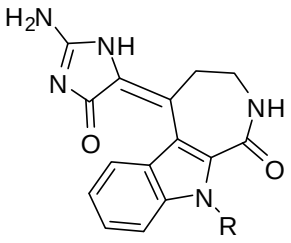


Taurodispacamides (**132**)

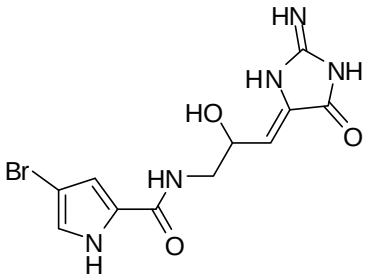
Taurodispacamide A (**132**) was isolated from the methanolic extract of sponge *Agelas oroides* collected off the bay of Naples.[32] It displayed good antihistaminic activity by completely abolishing 0.1 μM response of histamine in a reversible manner.

4.1.2.30 Spongiasidine:

Chemical investigations of an Okinawan sponge *Hymeniacidon* sp. collected off Ishigaki Islands, by Kobayashi and co-workers furnished pyrrolo[2,3-*c*]-azepine-type alkaloids spongiasidines A (**133**) and B (**134**).[105] Both inhibited cyclin dependent kinase 4 with IC_{50} values of 32 and 12 μg/mL, while they showed IC_{50} values of 8.5 and 6.0 μg/mL against c-erbB-2 kinase, respectively.



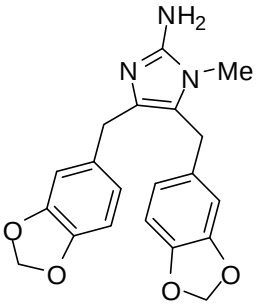
Spongiasidine A (**133**): R = Br
Spongiasidine B (**134**): R = H



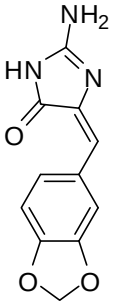
Mukanadin A (**135**)

4.1.2.31 Mukanadin A:

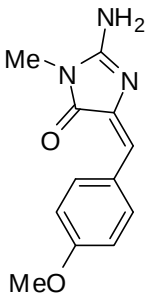
Mukanadin A (**135**) was isolated by Kobayashi's group from the extracts of Okinawan sponge *Agelas nakamurai* collected off Ie Island, Okinawa. It differs from monobromodispacamide in possessing a hydroxyl group at *C*-9.[103,106] Biological activity of Mukanadin A is not reported yet.



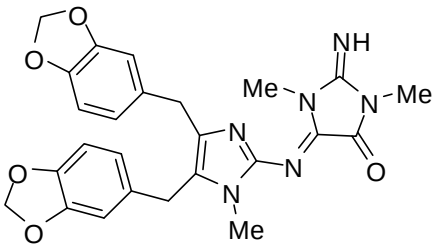
Leucettamine A (**136**)



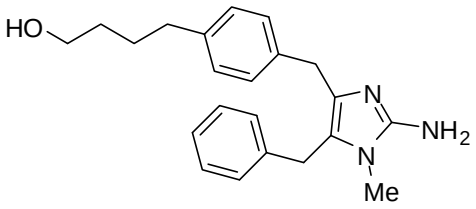
Leucettamine B (**137**)



Leucettamine C (**138**)



Leucettamidine (**139**)



(140)

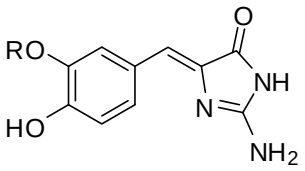
4.1.2.32 Leucettamines:

Leucettamines A (**136**), B (**137**) and leucettamidine (**139**) were isolated from Palaún sponge *Leucetta microraphis,*[107] while leucettamine C (**138**) was isolated from Fijian collection of calcareous sponge *Leucetta* sp.[108] Leucettamine A (**136**) binds to leukotriene B4 receptor as antagonist with Ki values 1.3, 100 and 5.3 μM, respectively.[109] It led to identification of leucettamine A as pure LTB4 antagonist, a new structural lead to inflammation therapy. Leucettamine A also showed good inhibition against gram positive bacteria *Staphylococcus aureus* and the fungus *Cladosporum herbarum*.[110] Effort were also made towards optimization of LTB4 antagonist activity of Leucettamine A by synthesizing the analogue which lacks symmetry but most of the synthetic analogue were less active, except **140** which has comparable activity to **136** with Ki = (2.4 ± 0.2).[111]

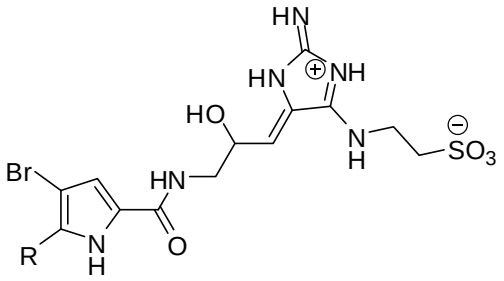
4.1.2.33 Polyandrocarpamines:

Chemical investigation of Fijian ascidian sponge *Polyandrocarpa* sp. resulted in isolation of polyandrocarpamine A (**141**) & B (**142**).[112] Polyandrocarpamine A was

selectively cytotoxic toward SF-268 (central nervous system) tumor cell lines with GI_{50} value of 65 μM, while B was less active with GI_{50} value of more than 80 μM.[113]



Polyandrocarpamine A (**141**): R = Me
Polyandrocarpamine B (**142**): R = H



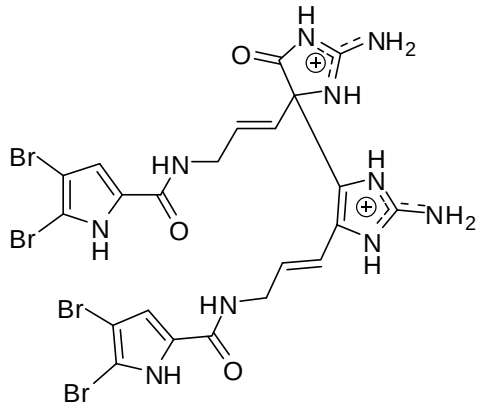
Tauroacidin A (**143**): R = Br, 9*S*/9*R* = 6:4
Tauroacidin B (**144**): R = H, 9*S*/9*R* = 1:1

4.1.2.34 Tauroacidin:

Tauroacidins A (**143**) and B (**144**), rare bromopyrrole alkaloids possessing a taurine residue, were isolated from extracts of an Okinawan sponge *Hymeniacidin* sp. Both tauroacidins A and B inhibited the enzymes epidermal growth factor receptor (EGFR) kinase and C-erb-2 kinase with IC_{50} value of 20 μg/mL each.[114]

4.1.2.35 Mauritiamine:

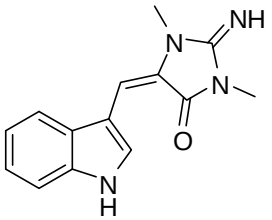
Keeping in view that chemical defense substances of sessile marine organism may act as potential non toxic antifouling agent, bioassay guided fractionation of methanolic extract of marine sponge *Agelas mauritiana*, collected off Hachijo-jima Island, Japan, yielded new antifouling agent mauritiamine (**145**). Mauritiamine showed inhibitory zone of 10 mm at 10 μg/disk against *Flavobacterium marinotypicum*. It also inhibited larval metamorphosis of barnacle *Balanus amphitrite* with ED_{50} value of 15μg/mL and was non toxic at conc. of 30 μg/mL.[22]



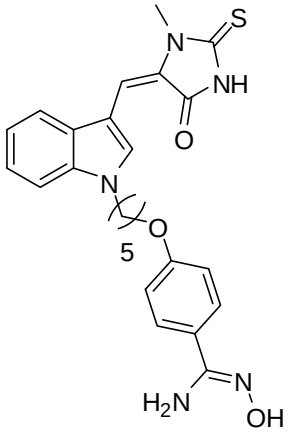
Mauritiamine (**145**)

4.1.2.36 Aplysinopsin:

Aplysinopsin (**146**), a marine natural product containing cyclic guanidine function was recently isolated from *Smenospongia aurea*,[115] earlier it has also been isolated from several other sources.[116] It acts on plasmepsin II and serotonin receptors as



Aplysinopsin (**146**)



(**147**)

dicationic molecule. Recently, we have discovered a thio analogue of aplysinopsin having potent antileishmanial activity with 10 times more activity and 401-folds less toxicity than standard drug pentamidine in cell based assays.[117]

4.1.3 Conclusion:

Leads from natural sources are better and more bio-friendly due to their co-evolution with the target sites in biological systems. As demonstrated in this review, a large number of 2-aminoimidazole and glycociamidine alkaloids isolated from marine environment have shown a range of biological activities, and may be treated as leads

for developing new compounds having antibacterial, antibiotic, antibiofilm formation, antiviral, antifungal, and anticancer activities.

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Chapter 4b

Chapter 4b: Isonaamine C and its analogues: Development of highly versatile, protecting group free synthesis and discovery of their antileishmanial and antibacterial potential

4.2.1 Introduction:

Marine sponges are still the main source of bioactive natural products.[1] Complex natural products isolated from marine sponges have been the basis for many clinical leads.[2] First marine drug, ziconotide (ω-conotoxin MVIIA),[3] isolated from a tropical marine cone snail, was approved in United States in 2004 for the treatment of chronic pain in spinal chord injury, under trade name Prialt. In October 2007, another marine natural product ET-743 (ecteinasidin-743/Yondelis/trabectedin), the antitumour compound from sea-squirt was approved by European Union for the treatment of soft tissue sarcoma.[4] Since the late 1980's, several marine alkaloids possessing either 2-aminoimidazole or 2-aminoimidazolinone moiety i.e. Isonaamine C (**1**), Leucettamine B (**2**), Oroidin (**3**), Hymenialdisine (**4**), and Dispacamide A (**5**) have been isolated from the genus *Leucetta* and many of them demonstrated interesting biological activities.[5] Recently, many efforts have also been devoted to discover the new biological activities of Oroidin and Hymenialdisine derivatives.[6]

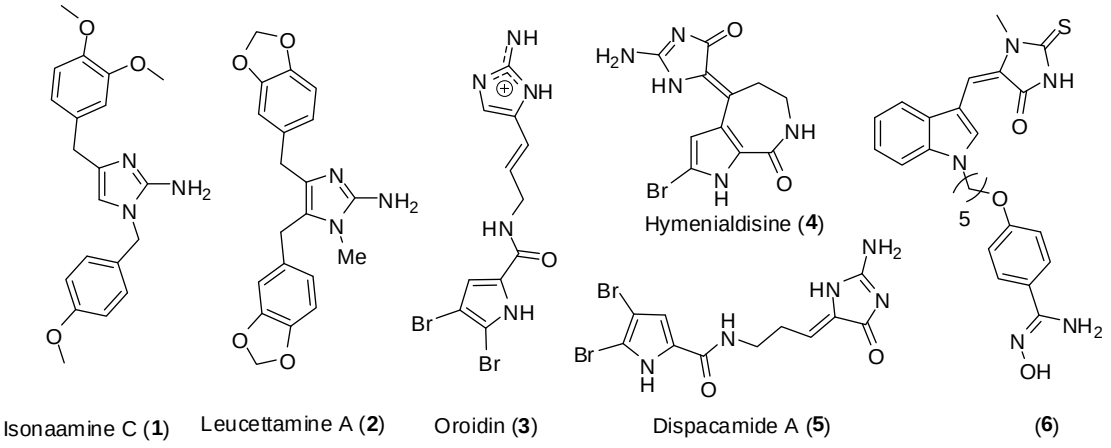
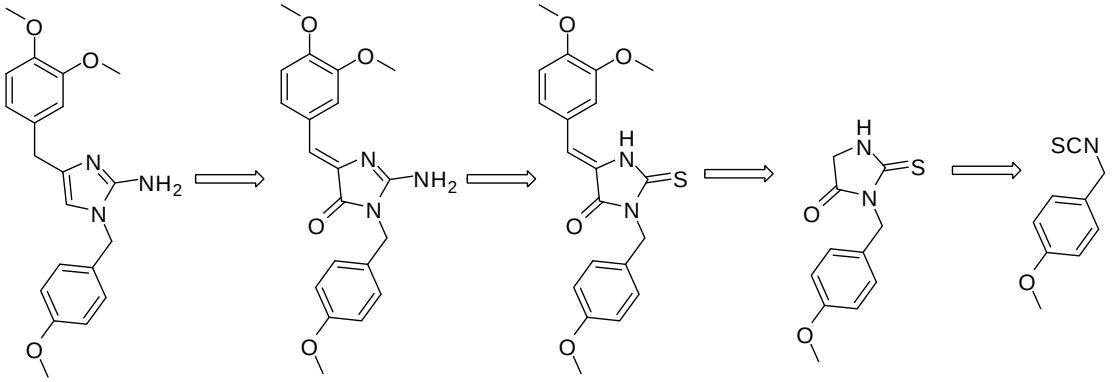


Figure 1. Some biologically important 2-aminoimidazole, 2-aminoimidazolinone, 2-thiohydantoin alkaloids.

As a part of our program to discover novel small molecule antiparasitic and anti-infective agents,[7] we recently reported the antileishmanial activity of aplysinopsin-pentamidine hybrid molecule.[8] Inspired by these findings, we focussed our attention on synthesis and bioevaluation of Isonaamince C and it's analogues as antileishmanial and antibacterial agents. Isonaamine C (**1**), a 2-aminoimidazole alkaloid, was isolated from marine sponge *Leucetta chagosensis* collected from Australian Bougainville Reef. Only biological activity known for this alkaloid is it's cytotoxicity against

HM02, HepG2, Huh7 tumour cell lines with GI_{50} values of 5.3, 2.2 and 2.1 μg/mL, respectively.[9]

Classical methods for synthesis of 2-aminoimidazoles can be divided in to two parts (i) starting from preformed imidazole ring and (ii) direct construction of 2-aminoimidazole ring. 2-Amino functionality can be introduced in imidazole ring by metallation followed by treatment with aryl azide and acid,[10] coupling with aryl diazonium salt and followed by reduction.[11] 2-aminoimidazole ring can also be directly constructed by reaction of α-haloketones and N-acetylguanidine,[12] α-aminocarbonyl compounds with cyanamide,[13] α-diketones with guanidine and subsequent reduction.[14] Among these, the most popular method is condensation of α-aminocarbonyl with cyanamide, but is very pH sensitive and can lead to self condensation of α-aminoaldehyde or ketone resulting into formation of symmetrical pyrazine.[15] In 1999, Molina *et al.* reported iminophosphorane mediated preparation of 2-amino-1,4-disubstituted imidazole from α-azidoesters, N-tosylisocyanate and amine leading to total synthesis of Isonaamine A, Dorimidazole A, and Preclathridine A, which involves tedious deprotection of 2-amino group by SmI_2.[16] We hypothesized that 2-amino-1,4-disubstituted imidazoles can be obtained by reduction of corresponding 2-aminoimidazolinones followed by dehydration, which in turn can be obtained by amination of corresponding 2-thiohydantoins (Scheme 1). This strategy looked very attractive to us, as it would also have provided us 2-thiohydantoins and 2- aminoimidazolinones analogues of Isonaamine C derivatives for bioevaluation against leishmanial and bacterial parasites.

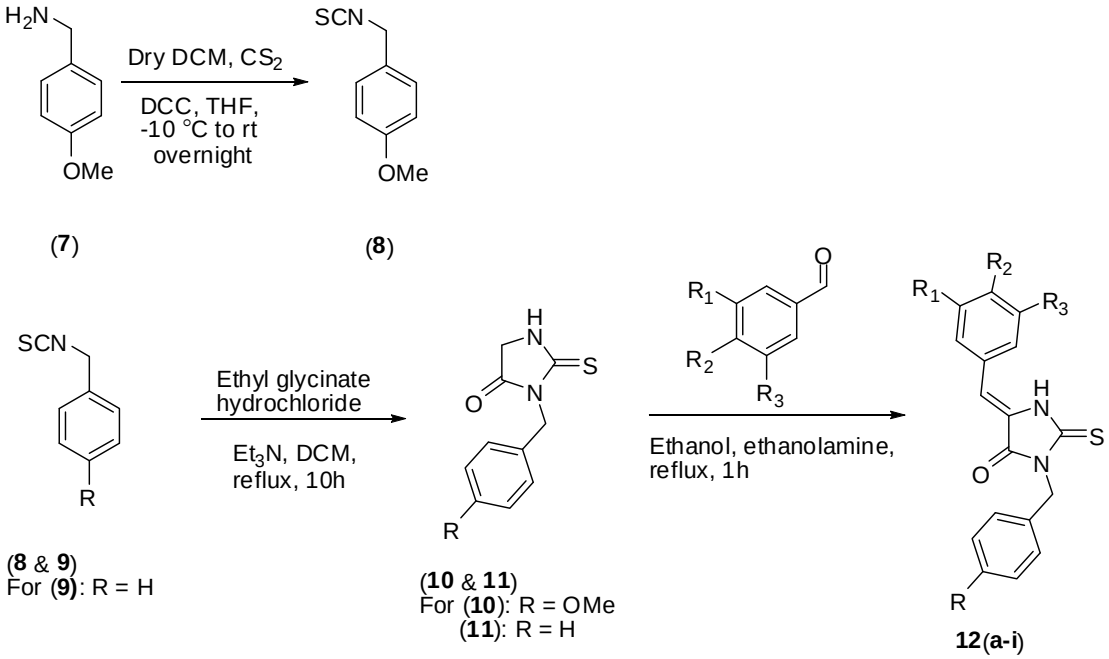


Scheme 1. Retrosynthesis of Isonaamine C.

In continuation of our efforts to discover novel small molecules as antiparasitic and anti-infective agents, we describe here an efficient, versatile and protecting group free synthesis of nine isonaamine C derivatives via corresponding 2-aminoimidazolinones, 2-thiohydantoin congeners and their biological evaluation.

4.2.2 Chemistry:

We started with the synthesis of 4-methoxybenzyl isothiocyanate (**8**) from 4-methoxybenzylamine (**7**) using the reported procedure[17] (Scheme 2). 3-(4-Methoxybenzyl)-2-thiohydantoin (**10**) was obtained by condensation of ethyl glycinate hydrochloride with 4-methoxybenzyl isothiocyanate (**8**) in 82% yield.



Scheme 2. Preparation of disubstituted 2-thiohydantoins.

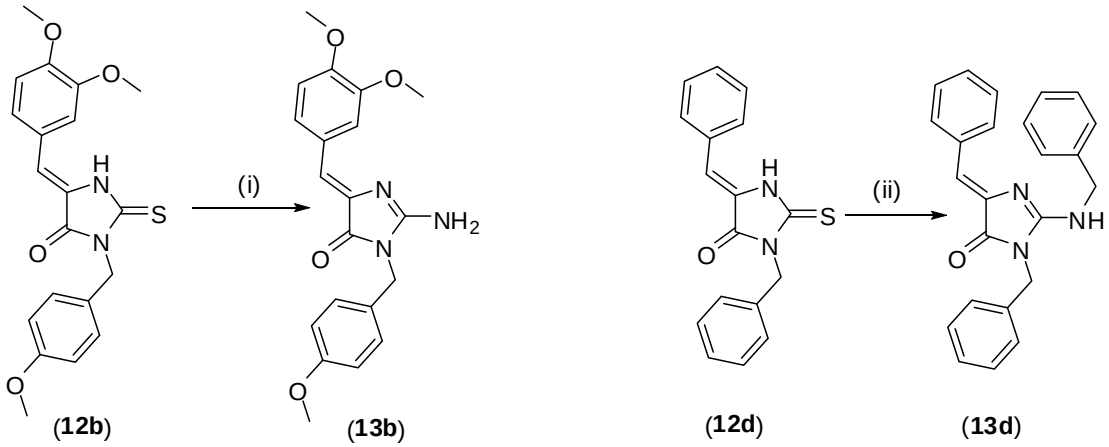
Condensation of commercially available benzyl isothiocyanate (**9**) with ethylglycinate hydrochloride afforded 3-benzyl-2-thiohydantoin (**11**) in 86% yield. 3-Substituted phenylmethylene-2-thiohydantoins **12**(**a**–**i**) were prepared by Knoevenegal condensation[18] of various benzaldehydes with 2-thiohydantoins (**10** and **11)**. (Scheme 2 and Table 1)

With these 3-benzyl substituted phenylmethylene-2-thiohydantoins **12**(**a**–**i**) in hand, we tried oxidative nucleophilic substitution of sulphur with aq. ammonia using tert-butyl hydroperoxide (TBHP)[19] as oxidizing agent taking (Z)-5-(3,4-dimethoxybenzylidene)-3-(4-methoxybenzyl)-2-thiohydantoin (**12b**) as the model

Table 1. 2-thiohydantoins **12**(**a**–**i**)

Compound No.	R	R_1	R_2	R_3	Yield (%)
12a	OMe	H	OMe	H	79
12b	OMe	H	OMe	OMe	76
12c	OMe	H	H	H	82
12d	H	H	H	H	85
12e	H	H	OMe	H	81
12f	H	H	Cl	H	84
12g	H	OMe	OMe	OMe	78
12h	H	H	OCH_2Ph	H	75
12i	H	OMe	OMe	H	84

substrate. But this resulted in a mixture of products with poor yield of desired amine **13b** (Scheme 3). To overcome this problem, it was thought that nucleophilic substitution of 2-thiomethyl group will be easier than sulphur and more importantly oxidizing agent will also be avoided, which may result in neat reaction.

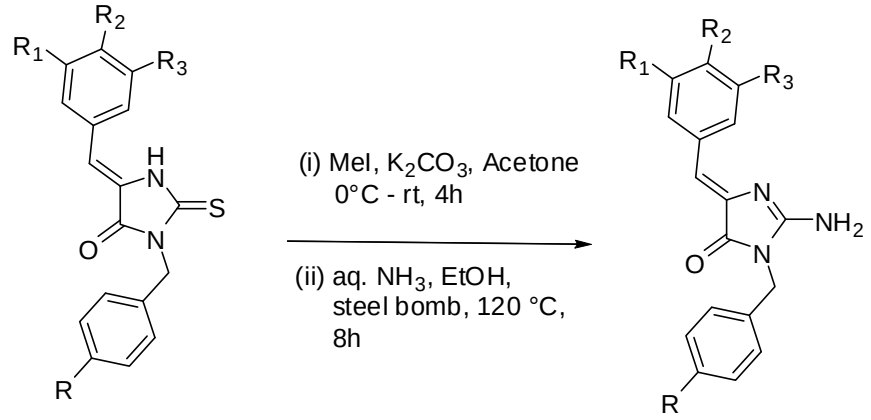


Reagents and conditions: (i) aq. NH_3, TBHP, MeOH, rt, 12h; (ii) (a) MeI, K_2CO_3, Acetone, 0°C - rt, 4h (b) Benzylamine, reflux, 8h.

Scheme 3. Preparation of 2-aminoimidazolinones by (a) oxidative nucleophilic substitution (b) by nucleophilic substitution of S-methyl derivative by benzylamine

In the mean time, S-methylation of **12d** was achieved by treating it with 1.1 equivalent of methyl iodide and K_2CO_3 in acetone. Refluxing the S-alkyl derivative with benzylamine furnished imidazolinone derivative **13d** in excellent yield (Scheme

3). Next, we tried amination of **12b** by heating at 120°C with 1:1 mixture of 30% aq. ammonia and ethanol in steel bomb. This reaction resulted in formation of a complex mixture of products. Overnight heating of **12b** in 4:1 mixture of 30% aq. ammonia and ethanol at 120 °C in steel bomb gave 2-aminoimidazolinone derivative **13b** in good yield (Scheme 4 and Table 2).



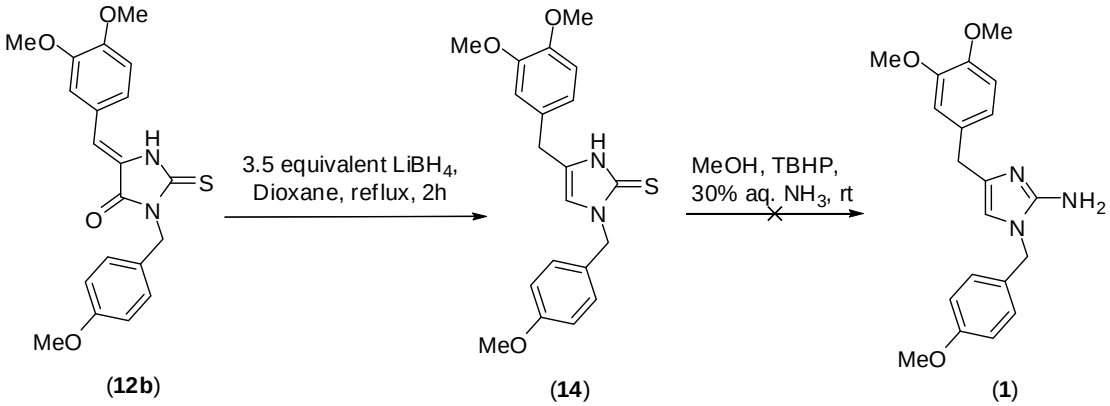
12(**a-i**) **13**(**a-i**)

Scheme 4. Synthesis of 2-aminoimidazolinones **13**(**a**–**i**) from corresponding 2-thiohydantoins **12**(**a**–**i**).

Table 2. 2-Amino-1,4-disubstituted imidazolinones (**13**(**a–c**) and **13**(**e–i**)

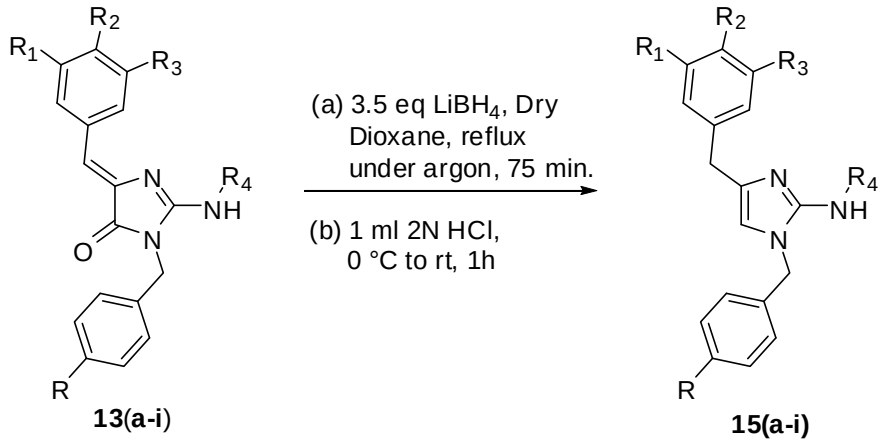
Compound No.	R	R_1	R_2	R_3	Yield (%)
13a	OMe	H	OMe	H	80
13b	OMe	H	OMe	OMe	77
13c	OMe	H	H	H	76
13e	H	H	OMe	H	74
13f	H	H	Cl	H	81
13g	H	OMe	OMe	OMe	77
13h	H	H	OCH_2Ph	H	82
13i	H	OMe	OMe	H	75

We also explored the possibility of direct amination of imidazo-2-thione (**14**) by oxidative nucleophilic substitution of 2-thio function in 2-thiohydantoin (**12a**) with ammonia. $LiBH_4$ reduction of 2-thiohydantoin resulted in the formation of imidazo-2-thione (**14**) in 67% yield which upon treatment with TBHP, aq. ammonia in methanol at rt didn't react to afford isonaamine C (**1**) (Scheme 5).



Scheme 5. An attempt toward conversion of 2-thiohydantoin to 2-aminoimidazole via imidazo-2-thione analogue.

To obtain the isonaamine C derivatives **15**(**a**–**i**) reduction of α,β-unsaturated amide function of **13**(**a–i**) and subsequent dehydration was planned. Initially lithium aluminium hydride (LAH) in dry THF was tried at various temperatures, but gave frustrating results. $LiBH_4$ is known to reduce the amidic carbonyl group, which otherwise is very difficult to reduce selectively. 2-Aminoimidazolinones on refluxing with 3 eq. of $LiBH_4$ in dioxane for 75 min. and subsequent treatment with 2N HCl furnished isonaamine C derivatives in 40-55% yields (Scheme 6 and Table 3). Although isonaamine C has been numbered as (**1**) in the introduction but in Table 3 and thereon it has been numbered as **15b** for ease of presentation.



Scheme 6. Direct reduction and dehydration of 2-aminoimidazolinones to isonaamine C analogues.

Table 3. Isonaamine C analogues **15**(**a-i**)

Compound No.	R_1	R_2	R_3	R_4	R_5	Yield (%)
15a	OMe	H	OMe	H	H	51
15b	OMe	H	OMe	OMe	H	42
15c	OMe	H	H	H	H	52
15d	H	H	H	H	CH_2Ph	55
15e	H	H	OMe	H	H	52
15f	H	H	Cl	H	H	55
15g	H	OMe	OMe	OMe	H	46
15h	H	H	OCH_2Ph	H	H	54
15i	H	OMe	OMe	H	H	48

4.2.3 Antileishmanial activity:

Antileishmanial evaluation of all 2-thiohydantoins **12**(**a–i**), 2-aminoimidazolinones **13**(**a–i**), 2-aminoimidazoles **15**(**a–i**) revealed that the isonaamine C analogues were more active than their corresponding 2-aminoimidazolinone and 2-thiohydantoins congeners. *N*-(4-methoxybenzyl) group at 1-position of 2-thiohydantoins was detrimental, while *N*-benzyl group was favoured for antiamastigote activity as demonstrated by percentage inhibition data of compound **12g**, **12i** and other 2-thiohydantoins against amastigotes. Compound **15d** showed only slightly lower % inhibition than **15e** but had much superior selectivity index of the latter against leishmania amastigotes. *N*,1,4-tribenzyl-1*H*-imidazol-2-amine (**15d**) showed best antileishmanial activity with IC_{50} value of 30.62 μM and selectivity index of 12.76. Selectivity index of compound (**15d**) is better than SSG (sodium stilbogluconate) and pentamidine, reference drugs. *In vivo* antileishmanial activity of most active compound (**15d)** is in progress.

Table 4. Antileishmanial activity of compounds (**12a** – **12i**), (**13a** – **13i**), (**15a** – **15i**)

Compound No.	Antiamastigote activity			
	% inhibition (at 40 μM)	IC_{50} (μM)	CC_{50} (μM)	Selectivity index
12g	72.99	ND	ND	ND
12i	72.97	14.96	31.54	2.11

13h	71.26	ND	ND	ND
15a	64.65	ND	ND	ND
15c	73.05	ND	ND	ND
15d	87.10	2.40	30.62	12.76
15e	95.70	8.71	13.49	1.55
15h	93.76	ND	ND	ND
15i	55.33	ND	ND	ND
SSG	--	71.90	398.77	6.38
Pentamidine	--	35.62	73.97	2.07

ND: not determined. [a]Selectivity index (SI) defined by the ratio CC_{50} (J774A.1 cells)/IC_{50} (Leishmania amastigotes). SSG: sodium stilbogluconate.

4.2.4 Antimicrobial activity:

Keeping in the view the antimicrobial activity of the 2-aminoimidazole and 2-aminoimidazolinone alkaloids all the synthesized 2-thiohydantoins **12**(**a**–**i**), 2-aminoimidazolinones **13**(**a**–**i**), 2-aminoimidazoles **15**(**a**–**i**) were also screened for their *in vitro* antimicrobial activity as tabulated in Table 5 along with Gentamicin, Ampicillin, Amphotericin B and Fluconazole used as a standard drugs.

Table 5. *In vitro* antimicrobial activity of compounds (**12a** – **12i**), (**13a** – **13i**), (**15a** – **15i**).

Compound No.	**MIC(µg/mL)[a]**									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
12g	>50	>50	>50	0.09	>50	>50	>50	>50	>50	>50
12i	>50	>50	>50	0.19	>50	>50	>50	50	>50	>50
13b	>50	>50	>50	6.25	>50	>50	>50	50	>50	>50
13e	>50	>50	>50	0.09	>50	>50	>50	>50	>50	>50
13f	>50	>50	>50	0.19	>50	>50	>50	50	>50	>50
13g	>50	>50	>50	0.045	>50	>50	>50	50	>50	>50
13h	>50	>50	>50	0.005	>50	>50	>50	>50	>50	>50
15a	>50	>50	>50	25	50	50	50	50	50	50
15b	>50	>50	>50	50	50	50	>50	>50	>50	50
15c	>50	>50	>50	50	50	50	50	50	>50	50
15d	>50	>50	>50	550	>50	>50	50	>50	>50	>50

15e	>50	>50	50	1.56	50	50	50	50	50	50
15f	>50	>50	12.5	3.12	50	50	25	12.5	25	50
15g	>50	>50	>50	1.56	>50	>50	50	>50	>50	>50
15h	>50	>50	50	0.19	50	50	50	25	>50	>50
15i	>50	>50	>50	0.19	>50	>50	>50	50	>50	>50
Gentamicin	0.78	0.78	0.39	0.78	-	-	-	-	-	-
Ampicillin	50	50	0.19	0.39	-	-	-	-	-	-
Amphotericin B	-	-	-	-	0.12	0.06	0.12	0.12	0.50	0.12
Fluconazole	-	-	-	-	0.50	1.0	2.0	1.0	2.0	1.0

1. E. coli (ATCC 9637), *2*. Pseudomonas aeruginosa (ATCC BAA-427), 3. Staphyloccus aerus (ATCC 25923), 4. Klebsiella pneumoniae (ATCC 27736). 5. *Cryptococcus neoformans, 6. Candida albicans*, 7. *Sporothrix schenckii, 8. Trichophyton mentagrophytes, 9. Aspergillus fumigates,* 10. *Candida parapsilosis* (ATCC-22019).

All the compounds having 4-methoxybenzyl substituent at *N*-1 were inactive both as antibacterial and antifungal agents. All the tested compounds were inactive against two bacteria *Escherichia coli*, *Pseudomonas aeruginosa*. Compound **13h** was most active against *Klebsiela pneumoniae*, a gram -ve bacteria, with MIC value of 0.005 µg/mL but its 2-thiohydantoin analogue **12h** was inactive while 2-aminoimidazole analogue **15h** was 38 times less active than **13h**. It clearly demonstrated that 2-aminoimidazolinone moiety was most favoured for antibacterial activity against *Klebsiela pneumoniae* in comparison to 2-thiohydantoin and 2-aminoimidazole ring systems. It was also supported by the fact that 3,4,5-trimethoxy derivative **13g** was also good antibacterial agent having MIC value of 0.045 µg/mL against the same bacteria while its 2-thiohydantoin and 2-aminoimidazole (**12g** and **15g**, respectively) analogues were less active. Trend of antibacterial activity of **13e**, **14e** and **15e** also supported the above mentioned finding. 4-chloro substituent was also favoured but was not as good as benzyloxy substituent, as compounds **13f** and **15f** were less active in comparison to **13h.** Both of these compounds were more active in comparison to reference drugs. A comparison of antibacterial activity of 2-aminoimidazolinones (**13a** – **13i**) revealed that methoxy and benzyloxy groups on benzyl ring at C-5 position of 2-amnoimidazolinone moiety. SAR of the antibacterial activity of synthesized compounds against *Klebsiela pneumoniae* is somewhat similar to that of antileishmanial activity.

4.2.5 Anticancer Activity:

Earlier report on moderate cytotoxicity of isonaamine C against two human cancer cell lines inspired us to screen these analogues (**15a** – **15i**) against a panel of human cancer cell lines including KB (oral squamous cell carcinoma), MCF-7 (breast cancer), A549 (lung carcinoma), C33A (cervical carcinoma), NIH3T3 (mouse embryo fibroblast). Compound **15f** having 4-chloro group at C-5 benzyl ring and unsubstituted benzyl ring at *N*-1 was found to be most active against all the tested cancer cell lines. **15f** also showed lowest IC_{50} value of 3.33 µg/mL against KB cells followed by C33A (4.73 µg/mL), A549 (7.61 µg/mL), and MCF-7 (10.27 µg/mL), respectively. It was also found to be 5.18 times more selective towards KB cells in comparison to that of NIH3T3 mouse embryonic fibroblast cells. Selectivity against all other cell lines was in the range of 3.6 – 1.7. Compound **15c** was cytotoxic against C33A cells with IC_{50} value of 8.85µg/mL and was 4.98 times more selective in comparison to NIH3T3 cells. Resulted tabulated in Table 5 also revealed that all other methoxy and benzyloxy group were not favoured for anticancer activity.

Table 6. Cytotoxicty of isonaamine C analogues **15(a** – **i**) against a panel of human cancer cell lines.

Compound No.	IC_{50}(µg/mL)[a]				
	KB	**MCF-7**	**A549**	**C33A**	**NIH3T3**
15a	8.95	25.69	12.61	13.28	33.14
15b	20.16	24.80	22.41	19.29	10.21
15c	31.17	26.84	32.81	8.85	44.12
15d	22.72	25.85	19.88	12.67	31.47
15e	44.04	>50	27.49	32.56	>50
15f	3.33	10.27	7.61	4.73	17.24
15g	>50	>50	45.69	37.69	>50
15h	13.12	16.09	12.89	9.32	29.65
15i	45.27	44.26	>50	31.63	>50

[a] KB (oral squamous cell carcinoma), MCF-7 (breast cancer), A549 (lung carcinoma), C33A (cervical carcinoma), NIH3T3 (mouse embryo fibroblast).

4.2.6 Conclusion:

A versatile, efficient and protecting group free route has been developed for synthesis of isonaamine C analogues. Our route has many advantages over previously reported methods. Some of the noteworthy advantages are: (a) convenient functionalization of C-4 benzyl group by simply using the appropriate benzaldehyde while method reported by Molina et al. needs appropriately substituted α-bromoester; (b) easy access to the 2-thiohydantoin and 2-aminoimidazolinone congeners of the isonaamine C derivatives; (c) tedious detosylation by SMI_2 has also been avoided; (d) better overall yields of the 2-aminoimidazoles starting from isothiocyanates. Antileishmanial and antibacterial screening revealed that *N*-benzyl group is advantageous for biological activity while it's 4-methoxy analogue is not favoured. Compound **15d** showed better *in vitro* antiamastigote activity than standard reference drugs SSG and Pentamidine by exhibiting 2 and 6 times higher values of selectivity indices, respectively. It was also established from antibacterial screening that 2-aminoimidazolinone moiety was most favoured for antibacterial activity as compound **13h** was most active against *Klebsiela pneumoniae*, a gram -ve bacteria, with MIC value of 0.005 μg/mL. Compound **13h** was 156 and 78 times more active than standard antibacterial drugs Gentamicin and Ampicillin, respectively. Anticancer screening against a panel of human cancer cell lines also led us to identification of 2-aminoimidazole analogue **15f** as moderate cytotoxic agent which was more active and selective anticancer agent in comparison to isonaamine C (**15b**) itself. It was also established that 4-methoxy group at C-1 of 2-aminoimidazole was detrimental for the anticancer activity of isonammine C analogues.

4.2.7 Experimental:

General Procedure for the synthesis of 3-substituted 2-thiohydantoins (10 – 11):

To a well stirred mixture of ethyl glycinate hydrochloride and 1.1 equivalent of Et_3N in DCM, 1 equivalent of benzyl isothiocyanate/4-methoxybenzyl isothiocyanate was added and refluxed for 10h. Then the reaction mixture was diluted with DCM, washed with water and organic layer was dried with anhyd. Na_2SO_4. Solvent was removed *in vacuo* and recrystallized from EtOH.

3-(4-Methoxybenzyl)-2-thiohydantoin (10):

Yield: 82%; mp 179-181°C; IR (KBr): 3458, 3207, 3007, 2949, 1738, 1611, 1535, 1512, 1431, 1351, 1291, 1243, 1171cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz): δ (ppm) 7.48 (d, 2H, J = 8.7 Hz), 7.10 (bs, 1H), 6.86 (d, 2H, J = 8.7 Hz), 4.96 (s, 2H), 4.06 (s, 2H), 3.80 (s, 3H); ^{13}C NMR ($CDCl_3$, 75MHz): 184.93, 171.27, 159.38, 130.61, 127.79, 111.38, 55.25, 48.40. 44.14. Anal. Calcd. for $C_{11}H_{12}N_2O_2S$: C 55.91, H 5.12, N 11.86; Found: C 55.68, H 5.24, N 11.83 %.

3-Benzyl-2-thiohydantoin (11) :

Yield: 86%; mp 172-174°C; IR (KBr): 3274, 3022, 2905, 1714, 1512, 1438, 1407, 1348, 1317, 1158cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz): δ (ppm) 7.46-7.43 (m, 2H), 7.31-7.7.24 (m, 3H), 4.97 (s, 2H), 4.01 (s, 2H); ^{13}C NMR ($CDCl_3$+DMSO-d_6), 75MHz): 184.91, 172.71, 136.24, 128.96, 128.76, 128.09, 44.57, 39.97. Anal. Calcd. for $C_{10}H_{10}N_2OS$: C 58.23, H 4.89, N 13.58; Found: C 58.05, H 4.72, N 13.46 %.

General Procedure for the synthesis of phenylmethylene-2-thiohydantoins 12(a–i):

1 equivalent of aromatic aldehyde and 1.2 equivalent of ethanolamine was added to a mixture of appropriate 2-thiohydantoin (**10** or **11**) in EtOH. Resulting mixture was refluxed for 1h and a yellow precipitate was obtained. Reaction mixture was cooled slowly to 0°C and filtered. Solid residue so obtained was washed with (10mL × 3) chilled alcohol. The crude product was crystallized from ethanol.

(*Z*)-3-(4-Methoxybenzyl)-5-(4-methoxybenzylidene)-2-thiohydantoin (12a):

Yield: 79%; mp 181-183°C; ESMS: 355 (M+1); IR (KBr): 3453, 3225, 2937, 2836, 1734, 1655, 1605, 1513, 1471, 1435, 1303, 1266, 1249, 1178cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz): δ (ppm) 8.82 (bs, 1H), 7.47 (d, 2H, J = 11.7 Hz), 7.38 (d, 2H, J = 11.7 Hz), 6.96 (d, 2H, J = 11.2 Hz), 6.84 (d, 2H, J = 11.8 Hz), 6.69 (s, 1H), 5.04 (s, 2H), 3.85 (s, 3H), 3.78 (s, 3H); ^{13}C NMR ($CDCl_3$+DMSO-d6, 75MHz): 178.68, 164.91, 150.89, 149.51, 136.33, 128.89, 128.77, 128.02, 125.92, 125.19, 124.78, 114.97, 113.23, 111.66, 56.64, 56.31, 44.69. Anal. Calcd. for $C_{19}H_{18}N_2O_3S$: C 64.39, H 5.12, N 7.90; Found: C 64.36, H 5.24, N 7.85 %.

(*Z*)-5-(3,4-Dimethoxybenzylidene)-3-(4-methoxybenzyl)-2-thiohydantoin (12b):

Yield: 76%; mp 196-198°C; ESMS: 385 (M+1); IR (KBr): 3454, 3195, 2937, 2837, 1733, 1657, 1599, 1526, 1474, 1436, 1355, 1266, 1221, 1136cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz): δ (ppm) 8.70 (s, 1H), 7.49 (d, 2H, J = 8.7 Hz), 7.05 (dd, 1H, J = 8.1, J' = 1.8 Hz), 6.94 (d, 1H, J = 8.4Hz), 6.89-6.85 (m, 3H), 6.69 (s, 1H), 5.06 (s, 2H), 3.95 (s, 3H), 3.93 (s, 3H), 3.81 (s, 3H); ^{13}C NMR ($CDCl_3$, 75MHz): 177.65, 163.63, 159.34, 150.71, 149.66, 130.49, 127.80, 125.68, 125.17, 122.27, 114.01, 113.87, 112.51, 111.77, 56.16, 56.06, 55.25, 44.23. Anal. Calcd. for $C_{20}H_{20}N_2O_4S$: C 62.48, H 5.24, N 7.29; Found: C 62.35, H 5.47, N 7.22 %.

(*Z*)-5-Benzylidene-3-(4-methoxybenzyl)-2-thiohydantoin (12c):

Yield: 82%; mp 185-187°C; IR (KBr): 3263, 2956, 1710, 1640, 1509, 1467, 1442, 1349, 1298, 1228, 1185cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz): δ (ppm) 8.90 (bs, 1H), 7.49-7.42 (m, 7H), 6.85 (d, 2H, J = 11.9 Hz), 6.73 (s, 1H), 5.04 (s, 2H), 3.78 (s, 3H); ^{13}C NMR ($CDCl_3$+DMSO-d6, 75MHz): 170.59, 156.94, 151.78, 134.50, 126.47, 124.18, 123.45, 122.92, 122.26, 120.65, 108.50, 108.36, 52.63, 41.71. Anal. Calcd. for $C_{18}H_{16}N_2O_2S$: C 66.64, H 4.97, N 8.64; Found: C 66.49, H 5.06, N 8.54 %.

(*Z*)-3-Benzyl-5-benzylidene-2-thiohydantoin (12d):

Yield: 85%; mp 224-226°C; IR (KBr): 3260, 2956, 1728, 1646, 1509, 1464, 1441, 1342, 1298, 1223, 1186cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz): δ (ppm) 7.72–7.69 (m, 2H), 7.36-7.24 (m, 8H), 6.60 (s, 1H), 4.99 (s, 2H); ^{13}C NMR ($CDCl_3$+DMSO-d6, 75MHz): 170.82, 157.24, 135.93, 133.14, 130.06, 129.77, 129.61, 129.11, 128.90, 128.29, 126.74, 114.30, 44.99. Anal. Calcd. for $C_{17}H_{14}N_2OS$: C 69.36, H 4.79, N 9.52; Found: C 69.08, H 5.01, N 9.34 %.

(*Z*)-3-Benzyl-5-(4-methoxybenzylidene)-2-thiohydantoin (12e):

Yield: 81%; mp 206-208°C; ESMS: 325 (M+1); IR (KBr): 3245, 2930, 1725, 1647, 1594, 1462, 1341, 1255, 1172cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz): δ (ppm) 8.90 (bs, 1H), 7.51 (d, 2H, J = 8.5 Hz), 7.48-7.24 (m, 5H), 6.96 (d, 2H, J = 8.7 Hz), 6.70 (s, 1H), 5.10 (s, 2H), 3.85 (s, 3H); ^{13}C NMR ($CDCl_3$+DMSO-d6, 75MHz): 183.42, 169.74, 165.93, 141.32, 137.44, 133.61, 133.50, 132.81, 130.34, 129.73, 119.63, 119.44, 60.59, 49.36. Anal. Calcd. for $C_{18}H_{16}N_2O_2S$: C 66.64, H 4.97, N 8.64; Found: C 66.55, H 5.12, N 8.59 %.

(*Z*)-3-Benzyl-5-(4-chlorobenzylidene)-2-thiohydantoin (12f):

Yield: 84%; mp 229-230°C; IR (KBr): 3453, 3225, 2937, 2836, 1734, 1655, 1605, 1513, 1471, 1435, 1303, 1266, 1249, 1178cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz): δ (ppm) 7.43-7.27 (m, 5H), 7.23 (m, 4H), 6.59 (s, 1H), 5.04 (s, 2H); ^{13}C NMR ($CDCl_3$+DMSO-d6, 75MHz): 179.49, 164.77, 136.79, 135.01, 132.66, 131.90, 129.60, 129.20, 128.45, 128.27, 127.29, 112.53, 44.61. Anal. Calcd. for $C_{17}H_{13}ClN_2OS$: C 62.10, H 3.98, N 8.52; Found: C 61.88, H 3.84, N 8.45 %.

(*Z*)-3-Benzyl-5-(3,4,5-trimethoxybenzylidene)-2-thiohydantoin (12g):

Yield: 78%; mp 178-180°C; ESMS: 385 (M+1); IR (KBr): 3401, 3102, 2994, 2839, 1710, 1636, 1576, 1499, 1455, 1422, 1362, 1318, 1251, 1194cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz): δ (ppm) 9.01 (bs, 1H), 7.52 (dd, 2H, J = 9.6 Hz, J' = 1.6 Hz), 7.37-7.29 (m, 3H), 6.68 (s, 1H), 6.62 (s, 2H), 5.12 (s, 2H), 3.90 (s, 9H); ^{13}C NMR ($CDCl_3$+ DMSO-d6, 75MHz): 178.07, 163.66, 153.72, 139.47, 135.50, 128.82, 128.55, 128.33, 127.98, 126.33, 114.11, 106.42, 61.11, 56.35, 44.72. Anal. Calcd. for $C_{20}H_{20}N_2O_4S$: C 62.48, H 5.24, N 7.29; Found: C 62.24, H 5.32, N 7.19 %.

(*Z*)-3-Benzyl-5-(4-(benzyloxy)benzylidene)-2-thiohydantoin (12h):

Yield: 75%; mp 199-201°C; ESMS: 401 (M+1); IR (KBr): 3429, 3038, 2916, 2868, 1727, 1654, 1600, 1510, 1463, 1429, 1378, 1342, 1302, 1244, 1182cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz): δ (ppm) 8.13 (d, 2H, J = 8.8Hz), 7.45-7.29 (m, 10H), 7.00 (d, 2H, J = 8.8 Hz), 6.97 (s, 1H), 5.12 (s, 2H), 4.79 (s, 2H); ^{13}C NMR ($CDCl_3$, 75MHz): 178.11, 164.43, 159.87, 136.32, 135.86, 134.63, 131.80, 128.64, 128.43, 128.16, 127.72, 127.46, 125.52, 124.73, 115.45, 114.20, 70.04, 44.42. Anal. Calcd. for $C_{24}H_{20}N_2O_2S$: C 71.98, H 5.03, N 6.99; Found: C 71.76, H 5.12, N 6.87 %.

(*Z*)-3-Benzyl-5-(3,4-dimethoxybenzylidene)-2-thiohydantoin (12i):

Yield: 84%; mp 182-184°C; IR (KBr): 3264, 2928, 1725, 1659, 1596, 1460, 1327, 1217, 1136cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz): δ (ppm) 8.98 (bs, 1H), 7.50 (m, 2H), 7.47-7.29 (m, 3H), 7.05 (dd, 1H, J = 7.3, J' = 1.8 Hz), 6.92-6.88 (m, 2H), 6.68 (s, 1H), 5.09 (s, 2H), 3.92 (s, 3H), 3.90 (s, 3H); ^{13}C NMR ($CDCl_3$, 75MHz): 178.11, 164.14, 151.13, 150.01, 135.95, 129.22, 128.95, 128.37, 126.03, 125.48, 122.88, 114.74, 112.96, 112.15, 56.56, 56.47, 45.14. Anal. Calcd. for $C_{19}H_{18}N_2O_3S$: C 64.39, H 5.12, N 7.90; Found: C 64.22, H 5.17, N 7.86 %.

General procedure for the synthesis of substituted 2-aminoimidazolinones 13(a–i):

To 500 mg of appropriate phenylmethylene-2-thiohydantoin in acetone at 0°C, 1.2 equivalent of MeI and K_2CO_3 (1.5 eq.) were added. The reaction mixture was slowly brought to room temperature and allow to stir for 4h. After TLC showed completion of reaction, solvent was removed in vacuo and resulting solid was washed with 100 mL of water. Dried thiomethyl derivative was added in the steel bomb containing 10 mL of ethanol and 50 mL of aqeuous ammonia. Reaction vessel was made air tight and heated at 120 °C for 8 h. Steel bomb was kept at 0°C for 5h. Crystallized 2-aminoimidazolinone analogue was filtered and residue was recrystallized from EtOH.

(*Z*)-2-Amino-1-(4-methoxybenzyl)-4-(4-methoxybenzylidene)-*1H*-imidazol-5(4*H*)-one (13a):

Yield: 80%; mp 219-220°C; ESMS: 338 (M+1); IR (KBr): 3378, 3020, 2931, 1672, 1599, 1476, 1439, 1363, 1254, 1153 cm^{-1}; ^1H NMR (DMSO-d_6, 300MHz): δ (ppm) 8.00 (d, 2H, J = 12.2 Hz), 7.49 (bs, 2H), 7.22 (d, 2H, J = 11.8 Hz), 6.90 (t, 4H, J = 10.6 Hz), 6.38 (s, 1H), 4.70 (s, 2H), 3.76 (s, 3H), 3.70 (s, 3H); ^{13}C NMR (DMSO-d_6, 75MHz): 170.17, 159.26, 159.04, 158.79, 138.99, 132.22, 129.35, 129.08, 128.96, 114.34, 114.29, 113.41, 55.58, 55.52, 41.77. EI-HRMS *m/z* Calcd. for $C_{19}H_{19}N_3O_3$ $[M]^+$ 337.1426; measured 337.1436.

(*Z*)-2-Amino-4-(3,4-dimethoxybenzylidene)-1-(4-methoxybenzyl)-*1H*-imidazol-5(4*H*)-one (13b):

Yield: 77%; mp 176-178°C; ESMS: 368 (M+1); IR (KBr): 3401, 3021, 1716, 1663, 1608, 1513, 1474, 1435, 1330, 1216, 1157cm^{-1}; ^1H NMR ($CDCl_3$, 300MHz): δ (ppm) 7.69 (s, 1H), 7.38 (dd, 1H, J = 8.4, J' = 1.6 Hz), 7.16 (d, 2H, J = 8.4 Hz), 6.86-6.79 (m, 3H), 6.75 (s, 1H), 4.70 (s, 2H), 3.87 (s, 3H), 3.85 (s, 3H), 3.76 (s, 3H); ^{13}C NMR ($CDCl_3$, 75MHz): 169.64, 159.56, 158.02, 149.65, 148.83, 136.56, 128.38, 128.30, 126.87, 124.66, 118.44, 114.61, 113.34, 110.98, 55.94, 55.87, 55.29, 42.50. EI-HRMS *m/z* Calcd. for $C_{20}H_{21}N_3O_4$ $[M]^+$ 367.1532; measured 367.1579.

(*Z*)-2-Amino-4-benzylidene-1-(4-methoxybenzyl)-*1H*-imidazol-5(4*H*)-one (13c):

Yield: 76%; mp 187-189°C; ESMS: 308 (M+1); IR (KBr): 3380, 3004, 1667, 1624, 1465, 1357, 1258, 1150cm^{-1}; ^1H NMR (DMSO-d_6, 300MHz): δ (ppm) 8.05 (d, 2H, J =

7.3 Hz), 7.67 (bs, 2H), 7.34 (t, 2H, J = 7.3 Hz), 7.23 (d, 3H, J = 8.6 Hz), 6.90 (d, 2H, J = 8.6 Hz), 6.40 (s, 1H), 4.73 (s, 2H), 3.71 (s, 3H); ^{13}C NMR (DMSO-d_6, 75MHz): 170.65, 159.98, 159.51, 141.39, 136.73, 131.02, 129.64, 129.50, 129.10, 128.23, 114.81, 113.27, 55.96, 42.28. EI-HRMS *m/z* Calcd. for $C_{18}H_{17}N_3O_2$ $[M]^+$ 307.1321; measured 307.1321.

(*Z*)-2-Amino-1-benzyl-4-(4-methoxybenzylidene)-*1H*-imidazol-5(4H)-one (13e):

Yield: 74%; mp 198-200°C; ESMS: 308 (M+1); IR (KBr): 3374, 2997, 1712, 1685, 1633, 1597, 1481, 1350, 1250, 1151cm^{-1}; ^{1}H NMR (DMSO-d_6, 300MHz): δ (ppm) 8.01 (d, 2H, J = 11.9 Hz), 7.50 (bs, 2H), 7.30-7.26 (m, 5H), 6.92 (d, 2H, J = 11.4 Hz), 6.39 (s, 1H), 4.79 (s, 2H), 3.76 (s, 3H); ^{13}C NMR (DMSO-d_6, 75MHz): 170.60, 159.73, 159.23, 139.35, 137.75, 134.61, 132.67, 129.56, 128.04, 127.84, 114.72, 113.98, 56.02, 42.75. EI-HRMS *m/z* Calcd. for $C_{18}H_{17}N_3O_2$ $[M+1]^+$ 308.1399; measured 308.1384.

(*Z*)-2-Amino-1-benzyl-4-(4-chlorobenzylidene)-*1H*-imidazol-5(4*H*)-one (13f):

Yield: 81%; mp 209-211°C; ESMS: 312 (M+1); IR (KBr): 3385, 3092, 1716, 1680, 1631, 1565, 1474, 1408, 1367, 1271, 1153cm^{-1}; ^{1}H NMR (DMSO-d_6, 300MHz): δ (ppm) 8.09 (d, 2H, J = 8.6 Hz), 7.75 (bs, 2H), 7.41 (d, 2H, J = 8.6 Hz), 7.33 (d, 2H, J = 6.8 Hz), 7.30-7.25 (m, 3H), 6.40 (s, 1H), 4.82 (s, 2H); ^{13}C NMR (DMSO-d_6, 75MHz): 175.13, 165.05, 146.65, 142.30, 140.49, 137.26, 136.97, 134.18, 133.88, 133.02, 132.57, 116.43, 47.59. EI-HRMS *m/z* Calcd. for $C_{17}H_{14}$ ClN_3O $[M]^+$ 311.0825; measured 311.0833.

(*Z*)-2-Amino-1-benzyl-4-(3,4,5-trimethoxybenzylidene)-*1H*-imidazol-5(4*H*)-one (13g):

Yield: 77%; mp 201-203°C; ESMS: 368 (M+1); IR (KBr): 3423, 2996, 1715, 1667, 1614, 1575, 1472, 1365, 1237, 1131cm^{-1}; ^{1}H NMR (DMSO-d_6, 300MHz): δ (ppm) 7.54-7.49 (m, 4H), 7.31-7.23 (m, 5H), 6.38 (s, 1H), 4.81 (s, 2H), 4.03 (s, 6H), 3.79 (s, 3H); ^{13}C NMR (DMSO-d_6, 75MHz): 170.23, 159.13, 153.07, 140.13, 137.94, 137.26, 131.94, 128.99, 127.81, 127.36, 113.49, 108.54, 60.56, 56.36, 42.32. ESI-HRMS *m/z* Calcd. for $C_{20}H_{21}N_3O_4$ [M+1] 368.17290; measured 368.17629.

(*Z*)-2-Amino-1-benzyl-4-(4-(benzyloxy)benzylidene)-*1H*-imidazol-5(4*H*)-one (13h):

Yield: 82%; mp 206-208°C; ESMS: 384 (M+1); IR (KBr): 3367, 3027, 1712, 1684, 1633, 1593, 1478, 1353, 1289, 1238, 1152cm^{-1}; ^{1}H NMR (DMSO-d_6, 300MHz): δ (ppm) 8.03 (d, 2H, J = 8.7 Hz), 7.49-7.24 (m, 12H), 7.02 (d, 2H, J = 8.7 Hz), 6.40 (s, 1H), 5.12 (s, 2H), 4.80 (s, 2H); ^{13}C NMR (DMSO-d_6, 75MHz): 170.19, 158.85, 158.39, 139.04, 137.38, 137.34, 132.25, 129.18, 128.99, 128.90, 128.35, 128.27, 127.81, 127.42, 115.15, 113.48, 69.71, 42.32. Anal. calcd. for $C_{24}H_{21}N_3O_2$: C 75.18, H 5.52, N 10.96; Found: C 74.92, H 5.61, N 11.04 %. EI-HRMS *m/z* Calcd. for $C_{24}H_{21}N_3O_2$ $[M]^+$ 383.1634; measured 383.1632.

(*Z*)-2-Amino-1-benzyl-4-(3,4-dimethoxybenzylidene)-*1H*-imidazol-5(4*H*)-one (13i):

Yield: 75%; mp 158-160°C; ESMS: 338 (M+1); IR (KBr): 3397, 3000, 1679, 1627, 1583, 1472, 1328, 1256, 1150cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz): δ (ppm) 7.79 (s, 1H), 7.45 (d, 1H, J = 8.2 Hz), 7.37-7.27 (m, 7H), 6.86-6.82 (m, 2H), 4.82 (s, 2H), 3.90 (s, 6H); ^{13}C NMR ($CDCl_3$+CD_3OD, 75MHz): 169.91, 157.98, 149.62, 148.77, 137.24, 136.89, 135.05, 129.05, 128.12, 126.88, 124.73, 118.57, 113.11, 110.94, 55.79, 42.84. EI-HRMS *m/z* Calcd. for $C_{19}H_{19}N_3O_3$ $[M]^+$ 337.1426; measured 337.1428.

Typical procedure for the synthesis of compound (13d)

To 250 mg of (*Z*)-3-benzyl-5-benzylidene-2-thiohydantoin (**12d**) in 40 mL of acetone at 0°C, 1.1 equivalent of methyl iodide was added. Reacton mixture was slowly brought to rt and stirred for 4h. Then solvent was evaporated and resulted solid was washed with 100 mL of water and dried. Dried thioalkyl derivative was then refluxed in neat benzylamine for 6h. Benzylamine was evaporated under reduced pressure, resulting mixture was taken in 60 mL chloroform and washed with 2% HCl in water. Organic layer was dried with Na_2SO_4 and solvent was evaporated. Solid so obtained was purified by column chromatography on silica gel and was crystallized from alcohol.

(*Z*)-1-Benzyl-2-(benzylamino)-4-benzylidene-*1H*-imidazol-5(4*H*)-one (13d) :

Yield: 85%; mp 181-183°C; ESMS: 368 (M+1); IR (KBr): 3343, 3064, 2925, 1707, 1658, 1586, 1444, 1373, 1159cm^{-1}; ^{1}H NMR (DMSO-d_6, 300MHz): δ (ppm) 8.08 (d, 2H, J = 7.5 Hz), 7.38-7.21 (m, 13H), 6.46 (s, 1H), 4.88 (s, 2H), 4.65 (d, 2H, J = 5.7 Hz); ^{13}C NMR (DMSO-d_6, 75MHz): 170.27, 158.35, 140.32, 139.38, 137.07, 136.23,

130.79, 129.03, 128.79, 128.75, 128.04, 127.97, 127.86, 127.53, 127.20, 113.81, 45.04, 42.25. EI-HRMS *m/z* Calcd. for $C_{20}H_{21}N_3O_4$ $[M]^+$ 367.1732; measured 367.1757.

Typical procedure for synthesis of compound (14):

To a 50 mL oven dried two necked round bottomed flask, 250 mg of (**12b**), 15 mL dioxane and 3.3 equivalent of $LiBH_4$ was added under argon atmosphere. Reaction mixture was refluxed for 1h and cooled to 0°C. 5 mL of 5 N acetic acid was added to the reaction mixture and stirred for 2h at rt. Reaction solvent was removed under reduced pressure, neutralized with $NaHCO_3$ and extracted with $CHCl_3$. Purification of the resulting solid afforded compound (**14**).

4-(3,4-Dimethoxybenzyl)-1-(4-methoxybenzyl)-1H-imidazole-2(3H)-thione (14):

Yield: 67%; semisolid; ESMS: 371 (M+1); IR (KBr): 3386, 2926, 1607, 1513, 1431, 1354, 1216cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz): δ (ppm) 7.27 (d, 2H, J = 8.4 Hz), 6.88 (d, 2H, J = 8.4 Hz), 6.81-6.71 (m, 3H), 6.22 (s, 1H), 5.09 (s, 2H), 3.86 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 3.71 (s, 2H); ^{13}C NMR ($CDCl_3$, 75MHz): 159.45, 149.03, 147.99, 129.68, 129.27, 129.10, 128.02, 120.83, 114.19, 114.06, 111.98, 111.34, 111.24, 55.92, 55.29, 49.78, 30.89.

General procedure for direct reduction and dehydration of 2-aminoimidazolinones 15(a–i):

To a 50 mL oven dried two necked round bottomed flask, 150 mg of 2-aminoimidazol-4-one, 3.3 equivalent of $LiBH_4$, 15 mL of dry dioxane was added under argon atmosphere. The reaction mixture was refluxed for 75 minutes and then cooled to 0°C. To the cooled reaction mixture 3 mL of 2N HCl was added with stirring and temperature was slowly brought to room temperature in 1h. Solvent was removed under reduced pressure, reaction mixture was neutralized with saturated $NaHCO_3$ slolution and extracted with chloroform. Resulting solid was purified by column chromatography using neutral alumina as adsorbent and 0.2 % of (10% aq. NH_3) in DCM as eluent.

1,4-Bis(4-methoxybenzyl)-1*H*-imidazol-2-amine (15a):

Yield: 51%; semisolid; ESMS: 324 (M+1); IR (KBr): 2995, 2853, 1606, 1511, 1443, 1354, 1299, 1247, 1177 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz): δ (ppm) 7.20 (d, 2H, J =

8.5 Hz), 7.08 (d, 2H, J = 8.6 Hz), 6.89-6.81 (m, 4H), 6.15 (s, 1H), 4.74 (s, 2H), 3.80 (s, 3H), 3.78 (s, 3H), 3.72 (s, 2H); ^{13}C NMR ($CDCl_3$, 75MHz): 159.35, 157.92, 147.51, 139.28, 137.02, 132.22, 129.82, 128.28, 127.99, 114.40, 113.92, 113.75, 112.30, 55.30, 55.24, 48.10, 33.83. ESI-HRMS *m/z* Calcd. for $C_{19}H_{22}N_3O_2$ $[M+H]^+$ 324.1712; measured 324.1728.

4-(3,4-Dimethoxybenzyl)-1-(4-methoxybenzyl)-1*H*-imidazol-2-amine (15b):

Yield: 42; semisolid; ESMS: 354 (M+1); IR (KBr): 3386, 3021, 2926, 1607, 1513, 1431, 1354, 1216cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz): δ (ppm) 7.08 (d, 2H, J = 8.6 Hz), 6.89-6.76 (m, 5H), 6.17 (s, 1H), 4.75 (s, 2H), 3.84 (s, 6H), 3.79 (s, 3H), 3.72 (s, 2H); ^{13}C NMR ($CDCl_3$, 75MHz): 159.38, 148.78, 147.47, 147.33, 137.12, 132.86, 128.27, 128.05, 120.75, 114.40, 114.06, 112.36, 112.30, 111.24, 55.91, 55.78, 55.30, 48.11, 34.36. ESI-HRMS *m/z* Calcd. for $C_{20}H_{21}N_3O_4$ $[M+H]^+$ 354.18177; measured 354.17937.

4-Benzyl-1-(4-methoxybenzyl)-1*H*-imidazol-2-amine (15c):

Yield: 52%; semisolid; ESMS: 294 (M+1); IR (KBr): 3337, 2925, 2854, 1611, 1514, 1457, 1353, 1250, 1177cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz): δ (ppm) 7.28-7.16 (m, 5H), 7.07 (d, 2H, J = 8.6 Hz), 6.88 (d, 2H, J = 8.6 Hz), 6.17 (s, 1H), 4.73 (s, 2H), 3.81 (s, 3H), 3.74 (s, 2H); ^{13}C NMR ($CDCl_3$, 75MHz): 159.80, 147.99, 140.56, 139.70, 137.11, 129.29, 129.17, 129.04, 128.72, 128.39, 126.42, 114.84, 114.50, 114.40, 112.90, 55.73, 48.50, 35.21. EI-HRMS *m/z* Calcd. for $C_{18}H_{20}N_3O$ $[M+H]^+$ 294.16064; measured 294.16079.

***N*,1,4-tribenzyl-1*H*-imidazol-2-amine (15d):**

Yield: 55%; semisolid; ESMS: 354 (M+1); IR (KBr): 3332, 3030, 2923, 1588, 1445, 1355, 1159cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz): δ (ppm) 7.36-7.12 (m, 13H), 7.10 (d, 2H, J = 6.3 Hz), 6.13 (s, 1H), 4.81 (s, 2H), 4.49 (d, 2H, J = 5.7 Hz), 3.91 (s, 2H); ^{13}C NMR ($CDCl_3$, 75MHz): 149.48, 139.64, 138.06, 136.65, 129.50, 129.44, 128.92, 128.68, 128.36, 128.23, 127.71, 127.15, 126.40, 113.33, 48.88, 48.56. ESI-HRMS *m/z* Calcd. for $C_{24}H_{24}N_3$ $[M+H]^+$ 354.19702; measured 354.19685.

1-Benzyl-4-(4-methoxybenzyl)-1*H*-imidazol-2-amine (15e):

Yield: 52%; semisolid; ESMS: 294 (M+1); IR (KBr): 2924, 2854, 1599, 1442, 1354, 1219cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz): δ (ppm) 7.35-7.28 (m, 5H), 7.16 (d, 2H, J =

8.7 Hz), 6.83 (d, 2H, J = 8.7 Hz), 6.21 (s, 1H), 4.89 (s, 2H), 3.77 (s, 3H), 3.71 (s, 2H); ^{13}C NMR ($CDCl_3$, 75MHz): 159.67, 139.26, 132.37, 130.93, 129.84, 128.28, 128.09, 127.02, 126.86, 123.91, 123.39, 115.86, 114.07, 113.80, 112.39, 55.23, 48.58, 34.78. ESI-HRMS *m/z* Calcd. for $C_{18}H_{20}N_3O$ $[M+H]^+$ 294.16064; measured 294.15748.

1-Benzyl-4-(4-chlorobenzyl)-1*H*-imidazol-2-amine (15f):

Yield: 55%; semisolid; ESMS: 298 (M+1); IR (KBr): 3311, 3066, 1604, 1546, 1444, 1353cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz): δ (ppm) 7.28-7.02 (m, 9H), 6.08 (s, 1H), 4.72 (s, 2H), 3.63 (s, 2H); ^{13}C NMR ($CDCl_3$, 75MHz): 147.81, 138.64, 136.21, 135.98, 131.78, 130.22, 129.07, 128.40, 128.08, 126.85, 112.60, 48.57, 34.10. ESI-HRMS *m/z* Calcd. for $C_{17}H_{16}ClN_3$ $[M+H]^+$ 298.11174; measured 298.11110.

1-Benzyl-4-(3,4,5-trimethoxybenzyl)-1*H*-imidazol-2-amine (15g):

Yield: 46%; semisolid; ESMS: 368 (M+1); IR (KBr): 3424, 2998, 1718, 1668, 1612, 1571, 1472, 1365, 1241, 1132cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz): δ (ppm) 7.38-7.18 (m, 5H), 6.50 (s, 2H), 6.18 (s, 1H), 4.92 (s, 2H), 3.87 (s, 6H), 3.82 (s, 3H), 3.73 (s, 2H); ^{13}C NMR ($CDCl_3$, 75MHz): 153.54, 147.92, 139.70, 135.99, 135.59, 129.52, 128.66, 127.39, 114.50, 112.86, 106.19, 61.23, 56.44, 49.08, 34.25. EI-HRMS *m/z* Calcd. for $C_{20}H_{23}N_3O_3$ $[M]^+$ 353.1739; measured 353.1748.

1-Benzyl-4-(4-(benzyloxy)benzyl)-1*H*-imidazol-2-amine (15h):

Yield: 54%; semisolid; ESMS: 370 (M+1); IR (KBr): 3034, 2855, 1604, 1505, 1446, 1238, 1174cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz): δ (ppm) 7.33-7.15 (m, 7H), 7.07-7.03 (m, 5H), 7.01 (d, 2H, J = 6.3 Hz), 6.01 (s, 1H), 4.91 (s, 2H), 4.69 (s, 2H), 3.59 (s, 2H); ^{13}C NMR ($CDCl_3$, 75MHz): 157.23, 147.67, 137.23, 136.83, 136.00, 132.34, 129.84, 129.06, 128.54, 128.05, 127.89, 127.48, 126.87, 114.76, 112.45, 70.02, 48.53, 33.77. ESI-HRMS *m/z* Calcd. for $C_{24}H_{24}N_3O$ $[M+H]^+$ 370.19194; measured 370.19576.

1-Benzyl-4-(3,4-dimethoxybenzyl)-1*H*-imidazol-2-amine (15i):

Yield: 48%; semisolid; ESMS: 324 (M+1); IR (KBr): 3395, 2998, 1678, 1627, 1583, 1471, 1326, 1254, 1151cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz): δ (ppm) 7.35-7.12 (m, 6H), 6.86-6.82 (m, 2H), 6.20 (s, 1H), 4.82 (s, 2H), 3.83 (s, 3H), 3.77 (s, 3H), 3.71 (s, 2H); ^{13}C NMR ($CDCl_3$, 75MHz): 157.81, 149.21, 148.04, 139.69, 136.30, 132.87, 129.47,

128.98, 127.81, 127.27, 121.15, 114.49, 112.87, 112.63, 111.63, 56.30, 56.18, 48.95, 34.24. ESI-HRMS *m/z* Calcd. for $C_{19}H_{22}N_3O_2$ $[M]^+$ 324.17120; measured 324.17298.

Antiamastigote activity: Please see section **3.6.2.**

Cytotoxicity assay: Please see section **3.6.3.**

Antibacterial and antifungal evaluation:

The bacterial strains were grown on Sabroaud dextrose agar and nutrient agar media respectively. After the incubation fungal and bacterial growth were suspended in normal saline and maintained at $1.0–5.0\times10^3$ cfu/mL. The activity of compounds was determined by the NCCLS method for fungus using RPMI-1640 media buffered with MOPS (3-[*N*-morpholino]propanesulfonic acid) (Sigma-Aldrich Company) and Mueller Hinton broth for bacteria. The 96-well tissue culture plates were used for twofold serial dilution. The proper growth control, drug control and the blank were adjusted onto the plate. Compounds were dissolved in DMSO at a concentration of 1 mg/mL and 20 µL of this was added to 96-well tissue culture plate having 180 µL RPMI-1640 so the maximum concentration of the compound became 50 µg/mL. From here the solution was serially diluted resulting into the half of the concentration of test compounds and then inoculum was added and kept for incubation. Micro-titer plates were incubated at 35°C in a moist, dark chamber and MICs were recorded spectrophotometrically.

4.2.8 References:

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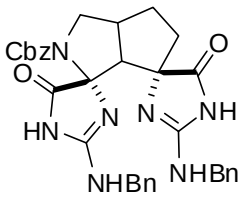
Chapter 5a

A one-pot chemoselective S-alkylation and acetylation of thiohydantoins using the alkyl orthoformate-$ZnCl_2$-Ac_2O reagent system

5.1.1 Introduction:

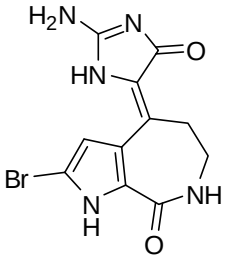
Thiohydantoins and their derivatives represent an important class of biologically active molecules having broad medicinal (anticancer,[1] anticovulsant,[2] antidiabetic,[3] antimicrobial,[4] antiarrythmic,[5] hypolipidemic[6] and hypotensive[7]) and agrochemical[8] (herbicidal and fungicidal) applications. Furthermore, many thiohydantoins are responsible for inhibition of fatty acid hydrolases,[9] glycogen phosphorylases,[10] amylases[11] and serine proteases.[12]

Thiohydantoins are useful synthons in natural product synthesis. Complex natural products such as the tetracyclic core of styloguanidine (**1**), and hymenialdisine (**2**), and bioactive heterocycles possesing a glycociamidine ring are commonly synthesized from their corresponding thiohydantoins. Conversion of 2-thiohydantoins to substituted glycociamidines is usually carried out in two steps; first thioalkyation of the thiohydantoin and then nucleophilic substitution of the thioalkyl group with a suitable nucleophile.[13-18] In general, thioalkylation is accomplished using alkyl halides which are toxic, dangerous, carcinogenic and non selective.[19-20] Hence, there remains a need for an efficient protocol for chemoselective *S*-alkylation of 2-thiohydantoins using surrogate alkyl halide reagents.



(**1**)

Tetracyclic core of the complex hexacyclic bisguanidine alkaloid styloguanidine.[13]



(**2**)

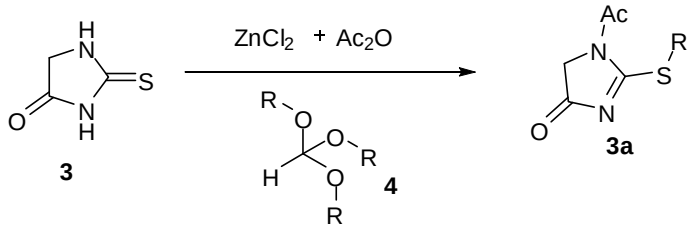
Hymenialdisine[14b]

Figure 1. Structures of the tetracyclic core of styloguanidine (**1**) and hymenialdisine (**2**).

Orthoesters are commonly used in the preparation of ketals and acetals.[21-23] However, in recent years, increased interest has focused on alkyl orthoformates as alternative reagents to alkyl halides for safer and selective alkylation protocols. In particular, Selva's group recently developed a one-pot procedure for highly selective mono-*C*-

methylation of arylacetonitrile using trimethyl orthoformate (TMOF) as the methylating agent.[24] Earlier, the same group reported that *O*-, *S*- and *C*- methylation of phenol, thiophenol and phenylacetonitrile, respectively, could be carried out using TMOF as the methylating agent.[25] Several TMOF-mediated *N*-methylations of aromatic amines and imidazole-like compounds have also been cited in the literature.[26-28]

In our endeavour to synthesize some key nitrogen heterocycles, it was observed that *S*-methylation and *N*–acetylation of 5-phenylmethylene-2-thiohydantoin (**5**) occured on treatment with trimethyl orthoformate in Ac_2O and $ZnCl_2$ in one-pot. In this chapter, we describe a one-pot chemoselective *S*-alkylation of 2-thiohydantoins and simultaneous acetylation of nucleophilic centres in the same molecule using alkyl orthoformates in Ac_2O and $ZnCl_2$. To the best of our knowledge, there have been no reports on this type of reaction.



Scheme 1. General scheme for the chemoselective *S*-alkylation and acetylation of 2-thiohydantoins.

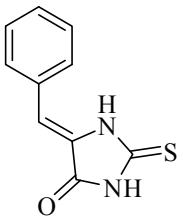
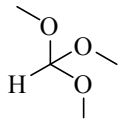
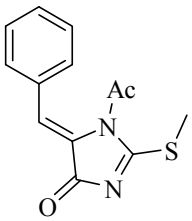
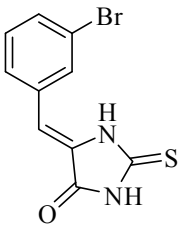
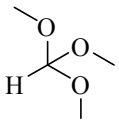
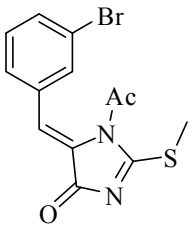
5.1.2 Results and Discussion:

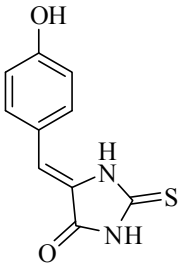
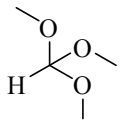
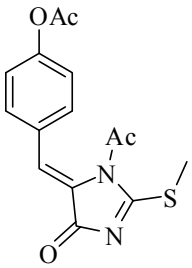
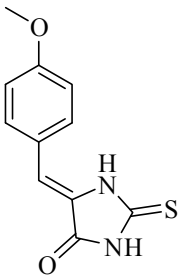
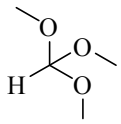
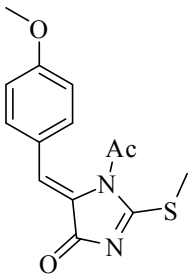
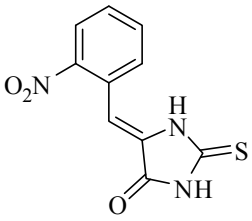
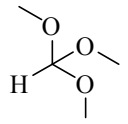
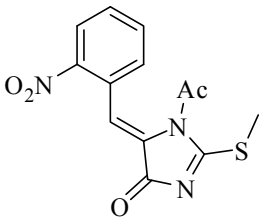
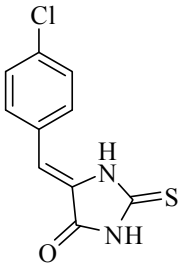
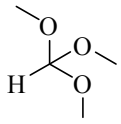
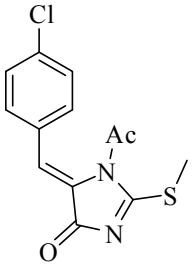
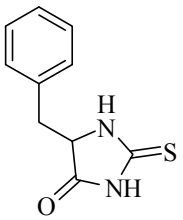
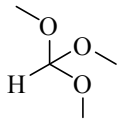
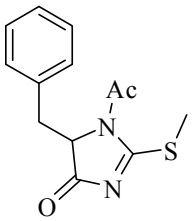
Initial investigations were focused on chemoselective *S*-alkylation of 5-substituted-2-thiohydantoins using 5-phenylmethylene-2-thiohydantoin (**5**) as the model substrate. At 100°C, 1 equivalent of $ZnCl_2$ was required for a 5:1 solution of trimethyl orthoformate (**4a**) and Ac_2O to convert completely 5-phenylmethylene-2-thiohydantoin (**5**) into its *S*-methyl *N*-acetyl derivative **5a**.

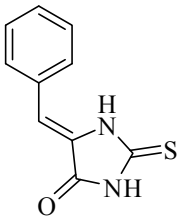
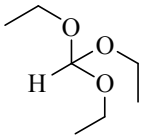
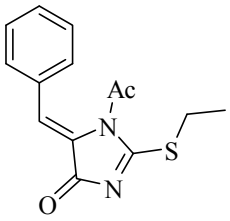
It was found that in the absence of either Ac_2O or $ZnCl_2$ the reaction failed to furnish the desired product. In order to explore whether substituents on the phenyl ring affected the reactivity of 5-phenylmethylene-2-thiohydantoins (PMHs), diversely phenyl substituted PMHs[29] were reacted with TMOF in Ac_2O and $ZnCl_2$. The results listed in Table 1 demonstrate that substituents on the phenyl ring do not affect the

reactivity of PMHs towards *S*-alkylation. 5-(2-Nitrobenzylidene)-2-thioxoimidazolidin-4-one (**9**) reacted surprisingly rapidly with alkyl orthoformates to give the highest yields of products. To investigate the chemoselectivity of the reagent system, we carried out the reaction of 5-(4-hydroxybenzylidine)-2-thiooxoimidazolidin-4-one (**7**) with TMOF in Ac_2O and $ZnCl_2$ which resulted in *N*-, *O*-acetylated, *S*-methylated product **7a**. TLC analysis of the reaction of 5-benzyl-2-thiohydantoin with reference compound (**12**) provided an insight into the reaction mechanism indicating that acetylation precedes alkylation. Based on the above observation, we propose that $ZnCl_2$ mediated acetylation is followed by nucleophilic attack of the *S*-nucleophile of 1-acetylated 2-thiohydantoin at the alkoxy carbon (not the carboxylic carbon) of the orthoformate resulting in S-alkylation (Scheme 2). This was supported by the fact that when triethyl orthoformate (TEOF) was used instead of TMOF, *S*-ethylation took place along with *N*-acetylation (entries 8-12).

Table 1. Chemoselective *S*-alkylation and acetylation of 2-thiohydantoins.

Entry	Substrate	Orthoformate	Product	Yield (%)	Time (h)
1	 **5**	 **4a**	 **5a**	78[a]	6
2	 **6**	 **4a**	 **6a**	72[b]	8

3	 **7**	 **4a**	 **7a**	69[b]	7
4	 **8**	 **4a**	 **8a**	71[a]	8
5	 **9**	 **4a**	 **9a**	88[a]	4
6	 **10**	 **4a**	 **10a**	65[a]	7
7	 **11**	 **4a**	 **11a**	82[b]	18

8				81[a]	6
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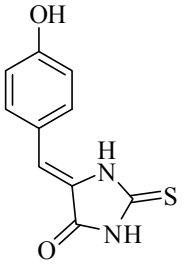
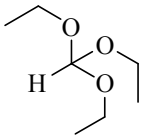
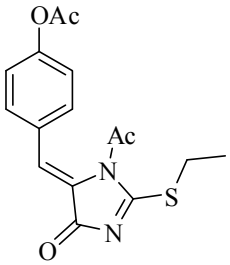
9				72[b]	7
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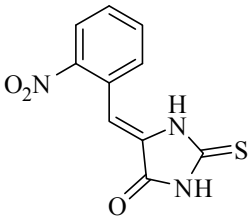
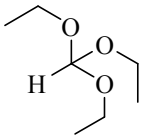
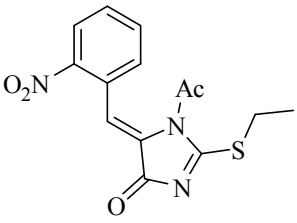
10				90[a]	4
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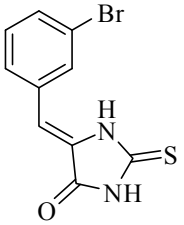
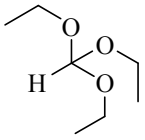
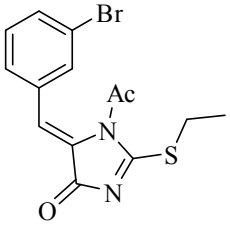
11				74[b]	8
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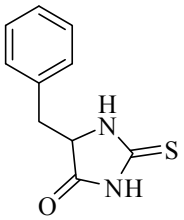
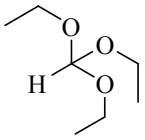
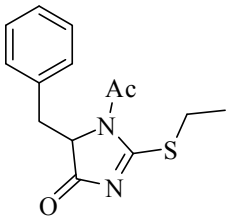
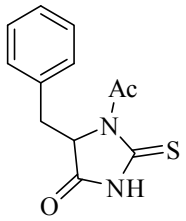
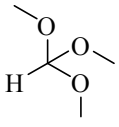
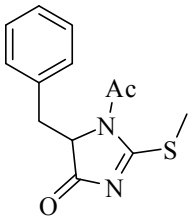
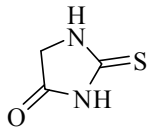
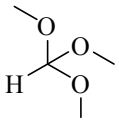
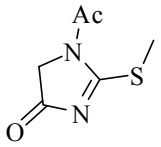
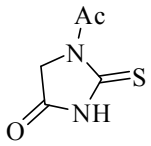
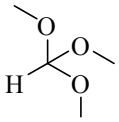
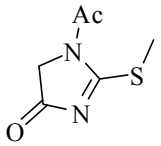
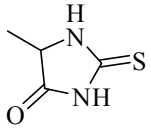
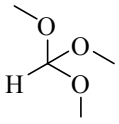
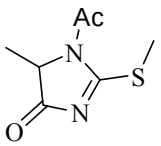
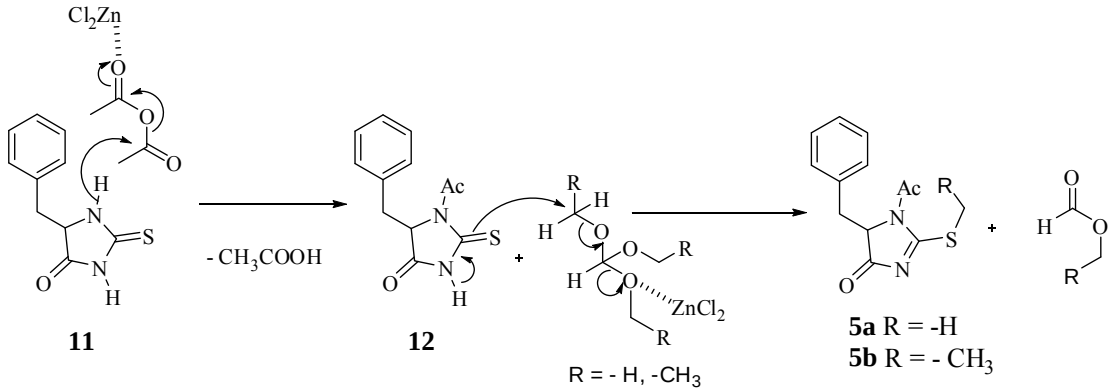
12				83[b]	18
	11	**4b**	**11b**		

13	 **12**	 **4a**	 **11a**	82[b]	15
14	 **3**	 **4a**	 **3a**	65[a]	18
15	 **13**	 **4a**	 **3a**	67[a]	18
16	 **14**	 **4a**	 **14a**	40[b]	16

a: purified by filtration. b: purified by column chromatography.

5-Benzyl (**11**) and 5-methyl-2-thiohydantoin (**14**) also gave the expected products (**11a**) and (**14a**), respectively, but were less reactive in comparison to their 5-methylene counterparts. Unsubstituted 2-thiohydantoin (**3**) yielded 1-acetyl-2-methylsulfanyl-4-imidazolidinone (**3a**) but the product of the reaction of 2-thiohyantoin (**3**) with TEOF in Ac_2O and $ZnCl_2$ was too unstable to be purified by column chromatography. 1-Methyl-2-thiohydantoin gave a complex mixture of products, while 1-acetyl-2-thiohydantoin (**13**) reacted smoothly to furnish 1-acetyl-2-methylsulfanyl-4-imidazolidinone (**3a**) in moderate yield. PMHs **5-10** gave better yields in comparison to 5-alkyl-2-thiohydantoins (**11**, **12** and **14**). Another interesting observation was that the active methylene group of 2-thiohydantoin (**3**) did not react with orthoformates, however, cyclopentendiones are known to react with orthoesters via their active methylene group.[30]



11 **12** **5a** R = -H **5b** R = - CH_3

Scheme 2. Proposed mechanism for the *S*-alkylation and acetylation of 2-thiohydantoins.

5.1.3 Conclusion:

In conclusion, we have developed a highly chemoselective one-pot *S*-alkylation (methylation, ethylation) and acylation protocol of thiohydantoins.[31] This new protocol should help to expedite the overall synthetic process and reduce the labour involved in total syntheses of natural products. This method could be used for derivatization of natural products for medicinal chemistry purposes as it chemoselectively alkylates the thio group whilst any other nucleophilic groups are acetylated. It may also be useful for the alkyaltion of thiohydantoin molecules containing oxidation prone functional groups, in which case oxidative nucleophilic substitution[14a] of the thio group will not be possible.

5.1.4 Experimental:

General Experimental Procedure:

$ZnCl_2$ (1.2 eq., 1.2 mmol) was added to a mixture of 2 mL of acetic anhydride and 10 mL of trialkyl orthoformate at 100 ºC. The resulting mixture was stirred for 5 minutes and then the 2-thiohydantoin (1 mmol) was added. The reaction was monitored by TLC analysis. After completion, the reaction mixture was cooled to room temperature and 20 mL of water was added. In most cases, a precipitate formed which was filtered and dried. In some cases (entries 2,3,11-13, and 16) a precipitate was not formed after addition of water. In these cases the reaction mixture was neutralized with saturated sodium bicarbonate solution and extracted with DCM (15 mLx3). The combined organic layers were dried over sodium sulfate and concentrated

in vacuo. The residue was purified by column chromatography using DCM as the eluent.

1-Acetyl-2-(methylthio)-1*H*-imidazol-4(5*H*)-one (3a):

Mp: decomposes at 120ºC; IR (KBr) ν = 1759, 1707, 1692, 1509, 1466, 1422, 1391, 1370, 1340, 1273, 1226 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 4.43 (s, 2H), 3.30 (s, 3H), 2.87 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 181.24, 169.73, 168.24, 50.24, 28.34, 27.36 ppm. Anal. Calcd for $C_6H_8N_2O_2S$: C 41.85, H 4.68, N 16.27 %. Found: C 41.47, H 4.72, N 16.22 %.

(*Z*)-1-Acetyl-5-benzylidene-2-(methylthio)-1*H*-imidazol-4(5*H*)-one (**5a**):

Mp 172-174 ºC; ESMS: *m/z* = 219 (M+1-Ac). IR (KBr) ν = 1747, 1712, 1706, 1634, 1597, 1495, 1371, 1291, 1215 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 8.18 (dd, 2H, J = 9.6 Hz, J' = 2 Hz), 7.46-7.43 (m, 3H), 7.00 (s, 1H), 2.67 (s, 3H), 2.64 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.74, 167.87, 163.17, 137.22, 134.27, 132.53, 130.80, 129.75, 129.55, 129.10, 126.04, 25.11, 14.89 ppm. Anal. Calcd for $C_{13}H_{12}N_2O_2S$: C 59.98, H 4.65, N 10.76 %. Found: C 59.89, H 4.60, N 10.68 %.

(*Z*)-1-Acetyl-5-(3-bromobenzylidene)-2-(methylthio)-1*H*-imidazol-4(5*H*)-one (**6a**): Mp 226-228 ºC; IR (KBr) ν = 1753, 1711, 1635, 1495, 1369, 1279, 1217 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 8.61 (d, 1H, J = 7.8 Hz), 8.01 (d, 1H, J = 7.8 Hz), 7.65 (t, 1H, J = 7.8 Hz), 7.53 (t, 1H, J = 7.8 Hz), 7.37 (s, 1H), 2.68 (s, 3H), 2.58 (s, 3H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 169.55, 167.48, 163.87, 137.68, 134.27, 132.54, 130.80, 129.83, 129.58, 129.10, 118.04, 25.24, 14.85 ppm. Anal. Calcd for $C_{13}H_{11}BrN_2O_2S$: C 46.03, H 3.27, N 8.26 %. Found C: 45.72, H 3.40, N 8.16 %.

(*Z*)-4-((1-Acetyl-2-(methylthio)-4-oxo-1*H*-imidazol-5(4*H*)-ylidene)methyl)phenyl acetate (7a): Mp 185-187 ºC; ESMS: *m/z* = 277 (M+1-Ac). IR (KBr) ν = 1750, 1723, 1638, 1598, 1490, 1374, 1279, 1206, 1167 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 8.20 (d, 2H, J = 8.7 Hz), 7.18 (d, 2H, J = 9 Hz), 6.96 (s, 1H), 2.67 (s, 3H), 2.62 (s, 3H), 2.34 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ = 169.38, 169.32, 167.34, 162.95, 151.92, 136.72, 133.30, 131.62, 124.28, 121.87, 24.68, 21.01, 14.47 ppm. Anal. calcd. for $C_{15}H_{14}N_2O_4S$: C 56.59, H 4.43, N 8.80 %. Found: C 56.52, H 4.40, N 8.73 %.

(*Z*)-1-Acetyl-5-(4-methoxybenzylidene)-2-(methylthio)-1*H*-imidazol-4(5*H*)-one (8a): Mp 197-199 ºC; ESMS: *m/z* = 309 (M+1-Ac). IR (KBr) ν = 1738, 1713, 1633, 1576, 1500, 1460, 1421, 1369, 1278, 1215 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.55 (s, 2H), 6.92 (s, 1H), 3.93 (s, 3H), 2.67 (s, 3H), 2.61 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.41, 169.29, 167.30, 153.09, 138.43 136.22, 129.34, 125.64, 109.39, 61.00, 56.00, 24.86, 14.50 ppm. Anal. Calcd for $C_{14}H_{14}N_2O_3S$: C 57.92, H 4.86, N 9.65 %. Found: C 57.64, H 4.81, N 9.57 %.

(*Z*)-1-Acetyl-2-(methylthio)-5-(2-nitrobenzylidene)-1*H*-imidazol-4(5*H*)-one (9a): Mp 185-187 ºC; IR (KBr) ν = 1751, 1699, 1562, 1419, 1387, 1346, 1311, 1216, 1128 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.61 (d, 1H, J = 7.8 Hz), 8.01 (d, 1H, J = 8.1 Hz), 7.65 (t, 1H, J = 8.1 Hz), 7.53 (t, 1H, J = 8.1 Hz), 7.37 (s, 1H), 2.68 (s, 3H), 2.58 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ = 168.99, 166.69, 165.92, 149.60, 139.36, 133.13, 132.64, 129.96, 128.21, 124.78, 118.07, 24.88, 14.60 ppm. Anal. calcd. for $C_{13}H_{11}N_3O_4S$: C 51.14, H 3.63, N 13.76 %. Found: C 50.93, H 3.76, N 13.72 %.

(*Z*)-1-Acetyl-5-(4-chlorobenzylidene)-2-(methylthio)-1*H*-imidazol-4(5*H*)-one (10a): Semisolid; IR (KBr) ν = 1744, 1712, 1633, 1589, 1496, 1370, 1281, 1215 cm^{-1}. ESMS: *m/z* = 253 (M+1-Ac). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.11 (d, 2H, J = 8.7 Hz), 7.42 (d, 2H, J = 8.4 Hz), 6.92 (s, 1H), 2.67 (s, 3H), 2.63 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.16, 168.92, 165.65, 138.72, 136.37, 133.21, 132.46, 129.07, 123.90, 24.89, 14.65 ppm. Anal. Calcd for $C_{13}H_{11}ClN_2O_2S$: C 52.97, H 3.76, N 9.50 %. Found: C 52.72, H 3.84, N 9.56 %.

(±) 1-Acetyl-5-benzyl-2-(methylthio)-1*H*-imidazol-4(5*H*)-one (11a):

Mp 172-174 ºC; ESMS: *m/z* = 221 (M+1-Ac); IR (KBr) ν = 1755, 1699, 1494, 1417, 1389, 1349, 1272, 1223 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.25 – 7.23 (m, 3H), 6.99 – 6.96 (m, 2H), 4.97 (dd, 1H, J = 5.4 Hz, J' = 2.4 Hz), 3.62 – 3.56 (m, 1H), 3.32 - 3.29 (m, 1H), 3.00 (s, 3H), 2.82 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 181.49, 171.91, 170.83, 133.72, 129.64, 128.97, 128.14, 62.61, 35.85, 28.36, 14.15 ppm. Anal. Calcd for $C_{13}H_{14}N_2O_2S$: C 59.52, H 5.38, N 10.68 %. Found: C 59.21, H 5.14, N 10.66 %.

1-Acetyl-5-methyl-2-(methylthio)-1*H*-imidazol-4(5*H*)-one (14a):

Mp 154-156ºC; IR (KBr) ν = 1759, 1707, 1477, 1374, 1345, 1297, 1216 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 4.45 (q, 1H, J = 6.9 Hz), 2.78 (s, 3H), 2.58 (s, 3H), 1.36 (d, 3H, J = 6.9Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 181.64, 173.55, 170.13, 58.87, 35.81, 27.52, 14.62 ppm. Anal. Calcd for $C_7H_{10}N_2O_2S$: C 45.15, H 5.41, N 15.04 %. Found: C 44.89, H 5.52, N 14.99 %.

(*Z*)-1-Acetyl-5-benzylidene-2-(ethylthio)-1H-imidazol-4(5*H*)-one (5b):

Mp 160-162 ºC; ESMS: *m/z* = 233 (M+1-Ac); IR (KBr) ν = 1748, 1712, 1636, 1596, 1494, 1371, 1290, 1215 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 8.17 (dd, 2H, J = 8.9 Hz, J' = 2 Hz), 7.45-7.42 (m, 3H), 6.98 (s, 1H), 3.26 (q, 2H, J = 7.2 Hz), 2.67 (s, 3H), 1.52 (t, 3H, J = 7.2 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.73, 167.85, 163.19, 137.19, 134.26, 132.55, 130.80, 129.77, 129.57, 129.11, 126.10, 25.46, 25.11, 14.19 ppm. Anal. Calcd for $C_{14}H_{14}N_2O_2S$: C 61.29, H 5.14, N 10.21 %. Found: C 60.92, H 5.10, N 10.15 %.

(*Z*)-1-Acetyl-5-(3-bromobenzylidene)-2-(ethylthio)-1*H*-imidazol-4(5*H*)-one (6b): Mp 210-212ºC; ESMS: *m/z* = 219 (M+1-Ac); IR (KBr) ν = 1751, 1713, 1665, 1596, 1492, 1413, 1356, 1279, 1216 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 8.01 (d, 1H, J = 7.5 Hz), 7.73 (s, 1H), 7.51 (d, 1H, J = 7.5 Hz), 7.33 (t, 1H, J = 8.1 Hz), 6.87 (s, 1H), 3.12 (q, 2H, J = 7.5 Hz), 2.57 (s, 3H), 1.56 (t, 3H, *J* = 7.5 Hz) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 169.66, 167.82, 163.45, 137.68, 134.16, 132.53, 130.28, 129.75, 129.54, 127.60, 118.54, 25.23, 21.37, 14.55 ppm. Anal. Calcd for $C_{14}H_{13}BrN_2O_2S$: C 47.60, H 3.71, N 7.93 %. Found: C 47.29, H 3.74, N 7.88 %.

(*Z*)-4-((1-Acetyl-2-(ethylthio)-4-oxo-1H-imidazol-5(4*H*)-ylidene)methyl)phenyl acetate (7b): Mp: 178-180 ºC; ESMS: *m/z* = 291(M+1-Ac); IR (KBr) ν = 1751, 1727, 1638, 1598, 1487, 1372, 1272, 1206, 1167 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 8.19 (d, 2H, J = 9 Hz), 7.18 (d, 2H, J = 8.7 Hz), 6.95 (s, 1H), 3.25 (q, 2H, J = 7.4 Hz), 2.67 (s, 3H), 2.35 (s, 3H), 1.50 (t, 3H, J = 7.2 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ = 168.83, 166.88, 162.14, 151.73, 136.60, 132.90, 131.45, 123.59, 121.80, 95.80, 25.48, 24.59, 20.82, 13.03 ppm. Anal. Calcd. for $C_{16}H_{16}N_2O_4S$: C 57.82, H 4.85, N 8.43%. Found: C 57.58, H 4.68, N 8.25%.

(*Z*)-1-Acetyl-2-(ethylthio)-5-(2-nitrobenzylidene)-1*H*-imidazol-4(5*H*)-one (9b): Mp 179-181 ºC; IR (KBr) ν = 1752, 1700, 1562, 1419, 1387, 1346, 1311, 1217 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.61 (d, 1H, J = 7.8 Hz), 7.99 (d, 1H, J = 8.1 Hz), 7.66 (t, 1H, J = 8.1 Hz), 7.52 (t, 1H, J = 8.1 Hz), 7.35 (s, 1H), 3.17 (q, 2H, J = 7.2 Hz), 2.65 (s, 3H), 1.44 (t, 3H, J = 7.2 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ = 169.38, 167.32, 167.03, 150.12, 139.45, 133.26, 132.64, 130.17, 128.28, 124.87, 118.11, 24.68, 21.22, 14.45 ppm. Anal. calcd. for $C_{14}H_{13}N_3O_4S$: C 52.66, H 4.10, N 13.16 %. Found: C 52.32, H 3.96, N 13.22 %.

(±) 1-Acetyl-5-benzyl-2-(ethylthio)-1*H*-imidazol-4(5*H*)-one (11b):

Mp semisolid; ESMS: *m/z* = 235 (M+1-Ac); IR (KBr) ν = 1742, 1704, 1465, 1351, 1290, 1221 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.24 – 7.21 (m, 3H), 7.00 – 6.97 (m, 2H), 4.92 (dd, 1H, J = 5.4 Hz, J' = 2.7 Hz), 3.68 (q, 2H, J = 7.3 Hz), 3.62 – 3.58 (m, 1H), 3.31 - 3.25 (m, 1H), 2.84 (s, 3H), 0.87 (t, 3H, J = 7.2 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 180.55, 171.22, 170.54, 133.28, 129.51, 128.54, 127.65, 61.81, 36.52, 28.08, 25.28, 14.56 ppm. Anal. Calcd for $C_{14}H_{16}N_2O_2S$: C 60.85, H 5.84, N 10.14 %. Found: C 60.66, H 5.92, N 10.16 %.

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Chapter 5b

$SnCl_2.2H_2O$: An efficient reagent for direct and selective oxidative desulfurization of phenylmethylene-2-thiohydantoins to corresponding hydantoins

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5.2.1 Introduction:

Hydantoins and their derivatives have long been the focus of study due to their numerous biological, therapeutic and agrochemical applications.[1] Recently, they have attracted more attention for their antidiabetic,[2] antiarrhythmics,[3] anticonvulsant,[4] antiallergic,[5] serotonin and fibrinogen receptor antagonists,[6] aldose reductase inhibitory[7] activities. Antitumor, antiangiogenic and antimetastatic[8] properties of hydantoins have also been reported. Hydantoin or related moieties are also found in many bioactive natural products e.g. Hydantocidin (**1**),[9] 4-Hydroxyphenylmethylene hydantoin (**2**),[8a] Agesamides (**3** & **4**),[10] Parazoanthin C (**5**),[11] and Axinohydantoins (**6, 7, 8, 9**).[12]

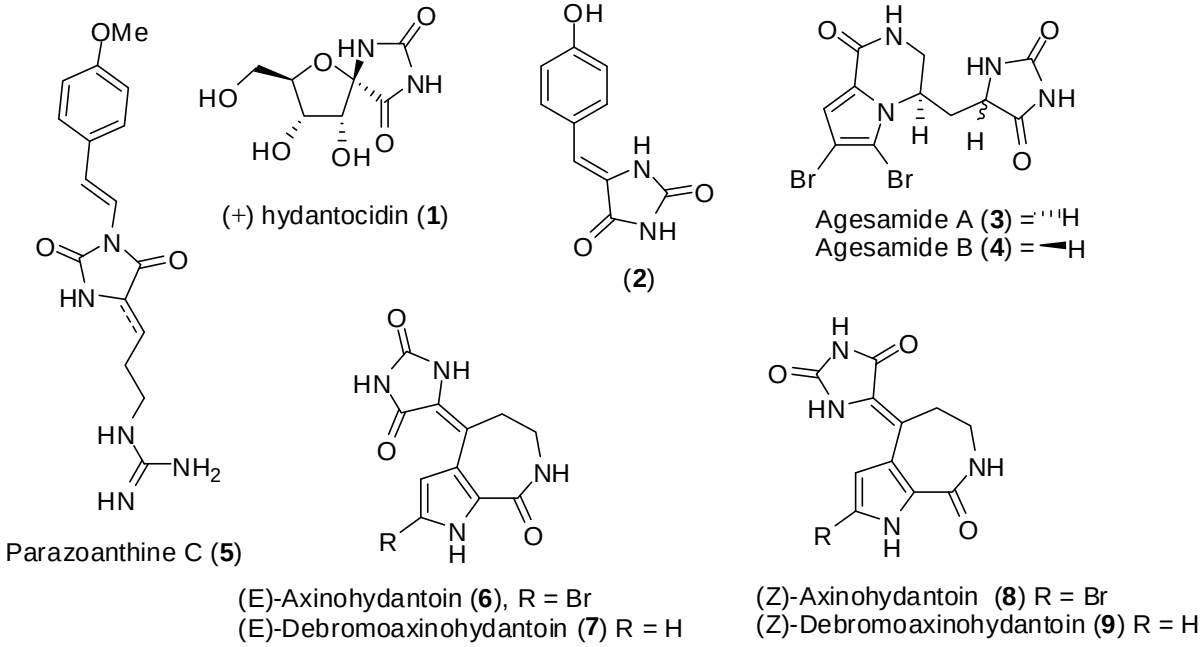


Figure 1. Bioactive hydantoin natural products.

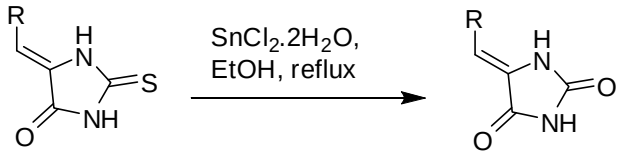
Desulfurization of 2-thiohydantoin for conversion to their corresponding hydantoins is still the commonly used method in synthesis of therapeutically relevant compounds and a lot of efforts have also been devoted towards development of new methodologies for synthesis of hydantoins. Existing methods for conversion of 2-thiohydantoins use $DMF/H_2O_2/CH_3COOH$,[13] $H_2O_2/NaHCO_3/CH_3CN$-H_2O,[14] NaOMe/MeI/DMF,[15] S-alkylation of 2-thiohydantoin followed by treatment with ethanolic HCl,[16] bromoacetyl benzene, and $Hg(OAc)_2$ with microwave.[17, 18] All these

methods suffer from no or poor selectivity, use of hazardous chemicals, oxidising agents (H_2O_2), or strong acids. Hence, there is a strong demand of an efficient reagent which directly converts the 2-thiohydantoins to their corresponding hydantoins selectively.

$SnCl_2.2H_2O$ is a good lewis acid with wide range of applications in synthetic organic chemistry. It catalyzes the deprotection of TBS,[19] esterification of oleic acid for biodiesel production,[20] synthesis of β-acetamidoketones and esters,[21] hydroperoxides,[22] hydroxyquinazolinones,[23] coumarins,[24] and benzodiazepines,[25] quinoxaline,[26] chromans,[27] dithiolanes,[28] allylation of aldehyde and ketones.[29] In our endeavour to synthesize some small molecule natural product mimics, we found that refluxing phenylmethylene-2-thiohydantoin with $SnCl_2.2H_2O$ in ethanol yields corresponding hydantoin derivative. Herein, we describe the selective desulfurization of phenylmethylene-2-thiohydantoins to corresponding hydantoins by $SnCl_2.2H_2O$. To the best of our knowledge, this kind of selective and direct conversion of phenylmethylene-2-thiohydantoins to hydantoins is not reported earlier.

5.2.2 Chemistry:

In an attempt to reduce the nitro group of o-nitrophenylmethylene-2-thiohydantoin (**10**), we discovered that $SnCl_2.2H_2O$ under refluxing condition desulfurizes it to the corresponding hydantoin (**10a**). Upon optimization, it was also found that 5 equivalent of $SnCl_2.2H_2O$ was required for complete conversion of compound (**10**) to (**10a**).



(**10-22**) (**10a - 22a**)

Scheme 1. General scheme for direct desulfurization of 2-thiohydantoins.

5.2.3 Results and Discussion:

Excited by this finding, we also tried the same with phenylmethylene-2-thiohydantoin (**11**) and obtained the corresponding hydantoin (**11a**) in 98% yield. (Scheme 1) To investigate whether two water molecules of $SnCl_2.2H_2O$ were essential for the

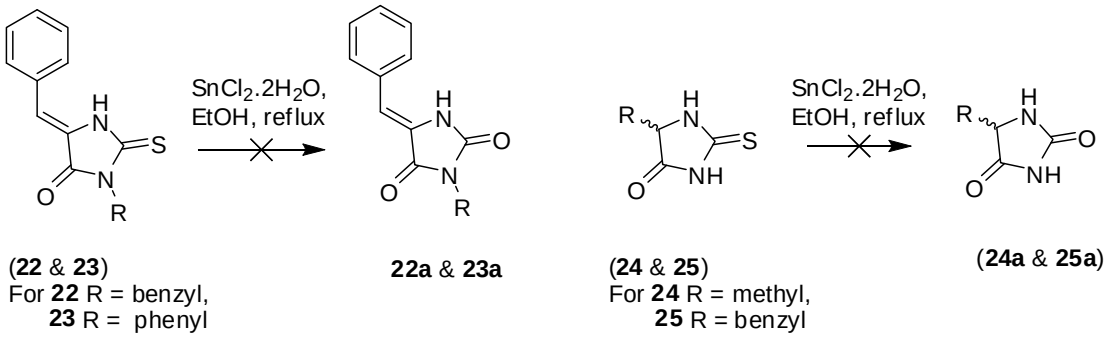
reaction, compound **11** was refluxed with anhydrous $SnCl_2$, but it failed to desulfurize the 2-thiohydantoin (**11)** to corresponding hydantoin (**11a**). Addition of 2 equivalents of water to the reaction mixture with anhydrous $SnCl_2$ also failed to start the reaction, indicating that water molecules of $SnCl_2.2H_2O$ were essential for the oxidative desulfurization activity of $SnCl_2.2H_2O$. To study the applicability of the reagent, a number of other substituted phenylmethylene-2-thiohydantoins (**12-21**) were subjected to oxidative desulfurization by $SnCl_2.2H_2O$ and results are summarized in Table 1. All the required substituted phenylmethylene-2-thiohydantoins were synthesized according to literature procedure.[30]

Table 1. Direct desulfurization of 2-thiohydantoins to their corresponding hydantoins.

Entry	R	Time (h)	Yield (%)
1	2-nitrophenyl (**10**)	8	95
2	Phenyl (**11**)	10	96
3	3-bromophenyl (**12**)	8	96
4	4-methylphenyl (**13**)	8	94
5	4-ethylphenyl (**14**)	8	95
6	4-propylphenyl (**15**)	8	95
7	4-chlorophenyl (**16**)	8	92
8	4-methoxyphenyl (**17**)	12	85
9	3,4-dimethoxyphenylphenyl (**18**)	12	82
10	2-bromophenyl (**19**)	8	91
11	4-benzyloxyphenyl (**20**)	12	84
12	Naphthyl (**21**)	8	90

Most of the phenylmethylene-2-thiohydantoins oxidatively desulfurized in excellent yields except those having methoxy substitutents (**17**, **18** and **20**) in the phenyl ring, which were little less reactive toward this reaction. Efforts towards desulfurization of 4-nitrophenylmethylene-2-thiohydantoin with $SnCl_2.2H_2O$ yielded complex mixture of products. Spectroscopic data of phenylmethylene hydantoins (**11a**, **12a** and **16a**) so obtained was identical to the reported data.[31] A few phenylmethylene 2-thiohydantoins having benzyl or phenyl substituents at *N*-3 i.e. compound **22** and **23**[32]

were also treated with $SnCl_2.2H_2O$, but were not converted to corresponding hydantoins. Similarly, 2-thiohydantoins having alkyl substituents at C-5 i.e. compound **24** and **25** were also not reactive toward desulfurization by $SnCl_2.2H_2O$, showing that it selectively desulfurizes the phenylmethylene-2-thiohydantoins.



(**22** & **23**)
For **22** R = benzyl,
23 R = phenyl

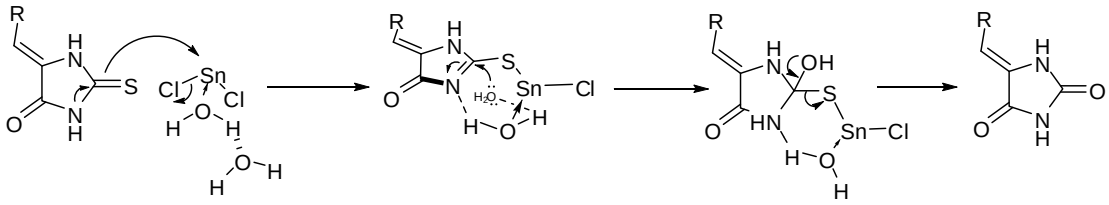
22a & **23a**

(**24** & **25**)
For **24** R = methyl,
25 R = benzyl

(**24a** & **25a**)

Scheme 2. Reactions of *N*-3 substituted phenylmethylene-2-thiohydantoins and C-5 alkyl substituted hydantoins.

Crystal structure of $SnCl_2.2H_2O$ shows that one of the two water molecules directly coordinate with Sn^{2+} ion while other water molecule remains hydrogen bonded to the first one and is sandwiched between the layers of $SnCl_2$.[33] It may however be possible that first nucleophilic displacement of chloro group of $SnCl_2$ by sulphur nucleophile of 2-thiohydantoin and then attack of the second water molecule may substitute the sulphur-complex with oxygen atom resulting in formation of corresponding hydantoin analogues.



Scheme 3. Proposed mechanism of oxidative desulfurization of 2-thiohydantoins by $SnCl_2.2H_2O$.

5.2.4 Conclusion:

In summary, $SnCl_2.2H_2O$ has been discovered as an efficient reagent for direct and selective desulfurization of phenylmethylene-2-thiohydantoins. Like existing literature methods, it does not involve the use of oxidising agents which makes it

applicable to the thiohydantoins having oxidation prone functional groups. Use of strong bases and mineral acids is also avoided making it amenable to base and acid sensitive substrates.

5.2.5 Experimental:

General Experimental Procedure: The mixture of 2-thiohydantoin (0.5 mmol, 1eq.), $SnCl_2.2H_2O$ (2.5 mmol, 5 eq.) in 30 mL ethanol was refluxed for 8 h. After TLC analysis showed completion of reaction, reaction mixture was cooled and a white precipitate was obtained. A white precipitate so formed was filtered and washed with (10 ml×3) chilled alcohol. Residue was crystallized from ethanol. In case of entry 9, where precipitate did not appear, reaction solvent was removed *in vacuo* and crude product was quickly column chromatographed on silica gel using chloroform as eluent.

(*Z*)-5-(2-Nitrobenzylidene)imidazolidine-2,4-dione (10a):

Mp: >250°C; IR (KBr) ν = 3423, 3218, 1763, 1711, 1638, 1539, 1343, 1273 cm^{-1}. ^1H NMR (300 MHz, CD_3OD): 7.74 – 7.70 (m, 1H), 7.56 – 7.46 (m, 4H), 6.62 (s, 1H) ppm; ^{13}C NMR (75 MHz, CD_3OD + DMSO-d_6): δ = 163.95, 154.53, 149.72, 130.17, 129.53, 128.67, 127.35, 126.33, 122.29, 109.25 ppm. Anal. Calcd. for $C_{10}H_7N_3O_4$: C 51.51, H 3.03, N 18.02 %. Found: C 51.32, H 3.10, N 18.11 %.

(*Z*)-5-Benzylideneimidazolidine-2,4-dione (11a):

Mp: 224-226°C; IR (KBr) ν = 3419, 3211, 3051, 1770, 1716, 1656, 1450, 1379, 1255 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ = 11.23 (s, 1H), 10.52, (s, 1H), 7.61 (d, 2H, *J* = 7.2 Hz), 7.42 -7.32 (m, 3H), 6.41 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 165.08, 155.22, 132.48, 128.88, 128.29, 127.89, 127.49, 107.84 ppm. Anal. Calcd. for $C_{10}H_8N_2O_2$: C 63.82, H 4.28, N 14.89 %. Found: C 63.64, H 4.42, N 14.97 %.

(*Z*)-5-(3-Bromobenzylidene)imidazolidine-2,4-dione (12a):

Mp: 241-243°C; IR (KBr) ν = 3440, 3145, 3042, 1779, 1730, 1661, 1470, 1382, 1246 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ = 11.36 (s, 1H), 10.65, (s, 1H), 7.70-7.66 (m, 2H), 7.42 (t, 1H, J = 7.6Hz), 7.29 – 7.23 (m, 1H), 6.51 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 164.75, 155.14, 132.38, 132.11, 129.71, 129.59, 129.54,

127.60, 123.57, 105.25 ppm. Anal. Calcd. for $C_{10}H_7BrN_2O_2$: C 44.97, H 2.64, N 10.49 %. Found: C 44.78, H 2.71, N 10.56 %.

(*Z*)-5-(4-Methylbenzylidene)imidazolidine-2,4-dione (13a):

Mp: >250°C; IR (KBr) ν = 3419, 3295, 3052, 1770, 1716, 1661, 1452, 1255 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ = 11.95 (s, 1H), 10.46, (s, 1H), 7.51 (d, 2H, *J* = 8.1 Hz), 7.21 (d, 2H, J = 8.1 Hz), 6.38 (s, 1H), 2.32 (s, 3H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 166.03, 156.09, 138.61, 130.57, 129.84, 129.82, 127.65, 108.98, 21.38 ppm. Anal. Calcd. for $C_{11}H_{10}N_2O_2$: C 65.34, H 4.98, N 13.85 %. Found C 65.18, H 5.01, N 13.94 %.

(*Z*)-5-(4-Ethylbenzylidene)imidazolidine-2,4-dione (14a):

Mp: >250°C; IR (KBr) ν = 3291, 1771, 1727, 1659, 1434, 1381, 1255 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ = 11.19 (s, 1H), 10.45, (s, 1H), 7.54 (d, 2H, *J* = 8.1 Hz), 7.23 (d, 2H, J = 8.1 Hz), 6.38 (s, 1H), 2.59 (q, 2H, J = 7.5 Hz), 1.18 (t, 3H, J = 7.5 Hz) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 166.05, 156.09, 144.91, 130.82, 129.91, 128.69, 127.69, 109.03, 28.45, 15.84 ppm. Anal. Calcd. for $C_{12}H_{12}N_2O_2$: C 66.65, H 5.59, N 12.96 %. Found: C 66.40, H 5.66, N 13.03 %.

(*Z*)-5-(4-Propylbenzylidene)imidazolidine-2,4-dione (15a):

Mp: 186-188°C; IR (KBr) ν = 3438, 3235, 2937, 1770, 1720, 1660, 1434, 1376, 1291, 1169 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ = 11.18 (s, 1H), 10.46, (s, 1H), 7.53 (d, 2H, *J* = 8.1 Hz), 7.21 (d, 2H, J = 8.1 Hz), 6.38 (s, 1H), 2.57 (t, 2H, J = 7.3 Hz), 1.65-1.63 (m, 2H), 0.89 (t, 3H, J = 7.3 Hz) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 165.05, 155.11, 142.27, 129.90, 128.85, 128.26, 126.74, 108.01, 28.47, 23.37, 13.08 ppm. Anal. Calcd. for $C_{13}H_{14}N_2O_2$: C 67.81, H 6.13, N 12.17 %. Found: C 67.64, H 6.11, N 12.15 %.

(*Z*)-5-(4-Chlorobenzylidene)imidazolidine-2,4-dione (16a):

Mp: >250°C; IR (KBr) ν = 3273, 2925, 1804, 1734, 1659, 1588, 1433, 1381 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 11.1 (s, 1H), 9.99 (s, 1H), 7.63 (d, 2H, *J* = 8.1 Hz), 7.42 (d, 2H, J = 8.1 Hz), 6.39 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 164.94, 155.19, 132.32, 131.43, 130.50, 128.22, 127.99, 106.33 ppm. Anal. Calcd. for $C_{10}H_7ClN_2O_2$: C 53.95, H 3.17, N 12.58 %. Found: C 53.88, H 3.22, N 12.60 %.

(*Z*)-5-(4-Methoxybenzylidene)imidazolidine-2,4-dione (17a):

Mp: 250-252°C; IR (KBr) ν = 3274, 1732, 1669, 1603, 1433, 1381 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 11.16 (s, 1H), 10.43 (s, 1H), 7.58 (d, 2H, *J* = 8.8 Hz), 6.95 (d, 2H, J = 8.8 Hz), 6.38 (s, 1H), 3.78 (s, 3H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 166.07, 159.91, 156.09, 131.54, 126.56, 125.91, 114.76, 109.14, 55.71 ppm. Anal. Calcd. for $C_{11}H_{10}N_2O_3$: C 60.55, H 4.62, N 12.84 %. Found: C 60.27, H 4.64, N 12.74 %.

(*Z*)-5-(3,4-Dimethoxybenzylidene)imidazolidine-2,4-dione (18a):

Mp: >250°C; IR (KBr) ν = 3023, 1732, 1669, 1636, 1437, 1381 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 11.17 (s, 1H), 10.63 (s, 1H), 7.04-7.81 (m, 3H), 6.38 (s, 1H), 3.78 (s, 6H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 166.08, 156.21, 149.82, 149.26, 126.60, 126.11, 113.07, 112.27, 109.69, 56.15, 56.02 ppm. Anal. Calcd. for $C_{12}H_{12}N_2O4$: C 58.06, H 4.87, N 11.29 %. Found: C 57.72, H 4.64, N 11.18%.

(*Z*)-5-(2-Bromobenzylidene)imidazolidine-2,4-dione (19a):

Mp: decomposes at 220°C; IR (KBr) ν = 3440, 3145, 3042, 1779, 1730, 1661, 1470, 1382, 1246, 1097, 1027 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ = 11.36 (s, 1H), 10.65, (s, 1H), 7.70 – 7.66 (m, 2H), 7.42 (t, 1H, J = 7.6 Hz), 7.29 – 7.23 (m, 1H), 6.51 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 164.75, 155.14, 132.38, 132.11, 129.71, 129.59, 129.54, 127.60, 123.57, 105.25 ppm. Anal. Calcd. for $C_{10}H_7BrN_2O_2$: C 44.97, H 2.64, N 10.49 %. Found: C 44.72, H 2.74, N 10.47 %.

(*Z*)-5-[4-(Benzyloxy)benzylidene]imidazolidine-2,4-dione (20a):

Mp: 240-242 °C; IR (KBr) ν = 3450, 3274, 3023, 1734, 1671, 1604, 1433, 1380 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 11.24 (s, 1H), 10.55 (s, 1H), 7.69-7.64 (m, 4H), 7.19-7.22 (m, 5H), 6.41 (s, 1H), 5.15 (s, 2H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 164.07, 155.74, 154.95, 140.69, 131.56, 129.30, 128.12, 127.75, 125.27, 123.54, 123.40, 115.25, 110.14, 68.7 ppm. Anal. Calcd. for $C_{17}H_{14}N_2O_3$: C 69.38, H 4.79, N 9.52 %. Found: C 69.04, H 4.66, N 9.60 %.

(*Z*)-5-(Naphthalen-1-ylmethylene)imidazolidine-2,4-dione (21a):

Mp: >250°C; IR (KBr) ν = 3422, 3146, 3041, 1776, 1728, 1663, 1382, 1230 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ = 11.23 (s, 1H), 10.52, (s, 1H), 7.61 (d, 1H, *J* = 7.2

Hz), 7.42 -7.32 (m, 6H), 6.41 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 164.75, 155.13, 132.73, 130.55, 129.75, 128.89, 128.19, 126.73, 126.36, 125.74, 125.24, 123.10, 103.95 ppm. Anal. Calcd. for $C_{14}H_{10}N_2O_2$: C 70.58, H 4.23, N 11.76 %. Found: C 70.45, H 4.22, N 11.64 %.

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List of Publications

1. One pot chemoselective S-alkylation and acetylation of thiohydantoins using the alkyl orthoformate-$ZnCl_2$-Ac_2O reagent system. **Ravi Kumar**, Prem M. S. Chauhan. *Tetrahedron Lett.* **2008**, *49*, 5475.

2. Synthesis of 2-(pyrimidin-2-yl)-1-phenyl-2,3,4,9-tetrahydro-1*H*-β-carbolines as antileishmanial agents. **Ravi Kumar**, Shahnawaz Khan, Aditya Verma, Saumya Srivastava, Preeti Viswakarma, Suman Gupta, Sanjeev Meena, Neetu Singh, Jayanta Sarkar, Prem M.S. Chauhan. *European Journal of Medicinal Chemistry,* **2009**, communicated

3. Synthesis and cytotoxicity evaluation of (Tetrahydro-β-carboline)-1,3,5-Triazine hybrids as antitumor agents. **Ravi Kumar**, Leena Gupta, Pooja Pal, Sanjay Babu Katiyar, Sanjeev Meena, Neetu Singh, Jayanta Sarkar, Sudhir Sinha, Arun Kumar Trivedi, Prem M. S. Chauhan. *European Journal of Medicinal Chemistry*, 2009, communicated.

4. $SnCl_2.2H_2O$ an effective reagent for direct oxidative conversion of thiohydantoins to hydantoins. **Ravi Kumar**, Sashi Pandey, Shahnawaz Khan, Prem M. S. Chauhan. *Tetrahedron Letters*, communicated.

5. Isonaamine C and it's analogues: Development of highly versatile, protecting group free synthesis and discovery of their antileishmanial and antibacterial potential. **Ravi Kumar**, Shahnawaz Khan, Suman Gupta, Prem M. S. Chauhan. Manuscript under preparation.

6. Marine 2-aminoimidazole, glycociamidine alkaloids and their synthetic analogues: New leads for drug development. **Ravi Kumar**, Shahnawaz Khan, Prem M. S. Chauhan. Manuscript under preparation